TECHNICAL REPORT
67-62-GP

RESISTANCE OF FLEXIBLE PACKAGING MATERIALS TO PENETRATION BY MICROBIAL AGENTS

by

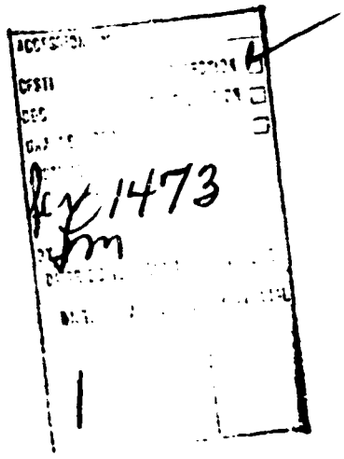
R. A. Lampi
General Engineering Laboratories
FMC Corporation
Santa Clara, California

Contract No. DA 19-129-AMC-662 (N)

April 1967

UNITED STATES ARMY
NATICK LABORATORIES
Natick, Massachusetts 01760

General Equipment
and Packaging Laboratory



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FOREWORD

The work on this contract was performed under Project 1M624101D552, 62141011, Packaging Exploratory Development, Task 02 - Design of Flexible Packaging Systems, Subtask on Packages for Processed Foods.

Microbial penetration of a container is one of the more important factors to be considered in evaluating its usefulness as a food container. A container for food products must have adequate penetration resistance to microorganisms that cause food spoilage, infection, or intoxication. Rigid metal cans have demonstrated that containers can be satisfactorily made to adequately protect foods from such harmful factors. The newly developed flexible package for processed foods consisting of a plastic-foil-plastic pouch has indicated it possesses this quality; however, more basic information was necessary to validate and confirm this claim. The logistic advantages of the flexible package for military use in combat feeding systems has provided the impetus to acquire supporting data.

The investigation described was performed in the Central Engineering Laboratories of FMC Corporation, Santa Clara, California. Dr. R. A. Lampi served as the Official Investigator. His collaborators were R. Griffin, H. Takahashi, and M. Nosroti.

Project Officer for the U. S. Army Natick Laboratories was Mr. Joseph W. Szczeblowski and the Alternate Project Officer was Mr. Gerald L. Schulz, both of the Packaging Division, General Equipment and Packaging Laboratory.

EDWARD A. NEBESKY, Ph.D.
Acting Director
General Equipment and Packaging Laboratory

APPROVED:

DALE H. SIELING, Ph.D.
Scientific Director

W. M. MANTZ
Brigadier General, USA
Commanding

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ABSTRACT

This project was conducted to study the resistance of flexible packaging materials used for thermally processed foods to penetration by microbial agents. Microbial agents were defined as bacterial cells being both aerobic and viable.

A procedure was evolved and equipment designed to study microbial penetration of flexible packaging materials.

The microbial penetration studies were made upon films, aluminum foils and laminates in sheet form. Creased films and laminates in sheet form and laminates in pouch form were subjected to abusive treatments.

Microbial penetration occurred only when pinholes were present in the materials. The 3-ply laminates approved for thermo processing when checked in sheet form, did not experience microbial penetration nor were pinholes present.

Creasing did not influence microbial penetration.

Laminates having polypropylene in their composition did not survive the abusive treatments.

I. INTRODUCTION

This is the third and final report on the work initiated under Quartermaster Corps Contract DA 19-129-AMC-662(N). The purpose of this project was to study the resistance to penetration by microbial agents of flexible packaging materials used for thermally processed foods. Microbial agents in this project are defined as bacterial cells being both aerobic and viable.

Three objectives of this project were:

1. Establishment of a test procedure for determining microbial penetration of flexible packaging materials and laminates made from three materials.

2. Determination of factors affecting bacterial penetration and whether or not test organisms can penetrate the individual materials and the laminated films.

3. Determination of the effect of simulated abuse of the packaging materials on susceptibility to microbial penetration.

II. EXPERIMENTAL

Before the experimental discussion, it may be appropriate here to mention the flexible packaging materials tested in this project. All tests were run with contract specified materials which were governed by the following specification:

> Materials and laminates are to withstand 250°F steam for 30 minutes without degradation and to meet Federal Food, Drug and Cosmetic Act Title 21 Section 121.2514 Resinous and polymeric coating.

	Film Material	Three Thicknesses of each Film Material (Thickness mils)*		
1.	Polyolefin	1.0	2.0	3.0
2.	Aluminum Foil	0.35	0.50	0.70
3.	Polyester	0.50	0.75	1.00

Five film laminates were selected as follows:

Laminate No.	Number of Plys	Film used in Laminate Material	Thickness of each ply (Thickness mils)*
1	2	Type "A Mylar" polypropylene A-22	0.50 1.50
2	2	Type "A Mylar" polypropylene A-22	0.50 2.00
3**	2	Type "A Mylar" Aluminum foil polypropylene A-22	0.50 0.35 1.50
4**	3	Type "A Mylar" Aluminum foil polypropylene A-30	0.50 0.35 3.00
5**	3	Polyester Aluminum foil Vinyl	0.50 0.35 3.00

*one mil=0.001 inches

**denotes the laminates used to make pouches in the abuse test

All the film laminates were adhesive bonded. Adhesive specifications were not available since sample laminates were furnished and adhesive composition is proprietary information.

The experimental work in this project can be grouped under two major phases. The first phase was concerned with the evaluation and development of test procedures to determine microbial penetration through films and laminates. The second phase involved the study of microbial penetration into filled pouches which were subjected to abusive conditions.

1. Development of Tests and Procedures

Examination for Pinholes

In the study of bacterial penetration through films, the question arose whether the penetration of microorganisms was through pinholes or through the material itself, and a reliable method for detecting pinholes needed to be established. A review of the available literature revealed two informal methods of testing for pinholes. Hartman, (1) described a test in which the test film is placed on a dry mixture of 98% sugar and 2% crystal violet dye and the film surface is flooded with water. Proctor and Nickerson (2) described a similar test but using agar as the base and dyeing the water with malachite green. Proctor and Nickerson also used white paper for the detection base. The above methods depend on the passage of water or aqueous dye solution through a pinhole and producing a colored spot upon the sugar dye mixture, or agar.

These two methods were evaluated using films and laminates punctured with 10 and 15 mil diameter needles and were found unsatisfactory for the following reasons:

- Consistency: consistent detection of all the pinholes could not be since water did not pass through all the punctured holes.

- Time: penetration times were found to vary from 15 minutes to 72 hours.

- Opaqueness: with opaque films, periodic inspection while conducting the pinhole test was not possible. Due to the varying penetration times, enough penetrant would pass through one pinhole to flood the entire detection area.

- Surface Contact: the test methods to be of value, require complete and intimate surface contact with the sugar dye mixture, or agar. This could only be achieved with thin individual films.

It was also observed during the course of the tests that all film surfaces exhibited non-wetting properties, and the non-wetting property may have resulted in the inconsistent penetration results. To reduce this non-wetting property, several wetting agents (TERGITOL Nonionic NPX, TERGITOL Nonionic TMN, TERGITOL Nonionic NP-27, Tergitol Anonic 7 [Union Carbide] and Sterex DJ Nonionic Monsanto) were added to the dyed solution. All the wetting agents facilitated the passage of the colored water through the punctured pinholes rapidly, and 100% detection was achieved. The addition of wetting agents on the other hand, produced an adverse effect of flooding on the detection surface which precluded the exact location of a pinhole.

Since the results obtained with Hartman's method and with Proctor and Nickerson's method were not wholly satisfactory, some modification in the detection method was necessary. From some additional tests, a satisfactory pinhole detecting procedure was evolved that was based on one of Proctor and Nickerson's techniques. In this method, white filter paper was used instead of the agar and pressure was used to aid in penetration. The filter paper was placed upon a flat, smooth surface and the test film was placed upon the filter paper. A piece of cotton soaked in a 0.01% aqueous crystal violet dye solution containing 100 ppm TERGITOL Nonionic TMN wetting agent was rubbed with moderate pressure back and forth across the film material to force the passage of the colored water through any pinholes and on to the filter paper. After examining for pinholes, the crystal violet dye solution was removed by rinsing with water.

This pinhole detection procedure when used on all films and laminates gave consistent and reliable results rapidly and was not affected by the non-wetting properties of the test film.

When handling samples of aluminum foil, it was found that any slight creasing or bending would produce pinholes. Because of the fragile property of the foil, rubbing across the foil surface was not possible and another pinhole detection method was necessary. This test was performed in a darkened room where pinholes were detected by passing the foil sample over a high intensity light beam.

2. The Development of Methods for Evaluating Microbial Penetration

Preliminary Work

The preliminary work in the development of methods for determining microbial penetration involved the examination of existing procedures used for hermetically sealed containers as well as for flexible packages. In the survey of methods for determining microbial penetration into hermetically sealed cans, several informal techniques have been used and are as follows:

a. The use of a bacillus organism capable of producing gas whereby penetration was measured by the presence or absence of gas formation.

b. The use of a fluorescein dye followed by the measurement of dye penetration by means of fluourometry.

c. Visual observation of bubble formation by techniques such as described by Le Febvre.(3)

d. Measurement of contamination by purposely contaminating cooling water with Coliform organisms and measuring penetration into cans.

e. Filling cans with distilled water, submerging into an acetic acid solution, and measuring conductivity to reveal penetration of the acid into the can. This technique has revealed that even a normal can leaks to the extent of permitting .001cc of acetic acid to enter the can.

f. The use of visible dyes in a manner similar to the technique with fluorescein dye.

A further check with the National Canners' Association revealed that they have no official standard method used for measuring susceptibility of metal containers to penetration by bacterial agents. The main concern with rigid containers is the evaluation of penetration through the seams and not through the material itself. Failures with rigid containers are for the most part concerned with the failures that occur with the mechanical closure.

The survey of microbial penetration studies with flexible packages revealed the use of two common methods: the "in-bag" technique and the "surface contact" technique.

The "in-bag" technique is characterized by the work of Hartman (1). Fill the bag with a liquid suspension of a test organism, place the bag into a flask containing a sterile liquid media and incubate the flask. This method is used to form the individual films or laminates into a bag. Positive penetration is observed by the growth of the test organism in the liquid media outside of the bag. This test procedure is a satisfactory one, but has some disadvantages. This method shows that microbial penetration has occurred but it does not locate the point of penetration. This technique also has a tendency to crease the material, which may be a factor in penetration. The detection of microbial penetration through a specified film area cannot be achieved.

The "surface contact" technique is characterized by the work of Proctor and Nickerson (2). This technique is to place the films or laminates in contact with a solid media on one side and a liquid suspension of the test organism on the other. After an incubation period, the film is removed and the solid media is incubated. The positive test for

penetration is the growth of the test organisms upon the solid media. One advantage of this method is that it locates the area of penetration.

Criteria for Design of Penetration Test Apparatus

The evaluation of existing methods to determine microbial penetration into cans and flexible pouches revealed that none of the procedures would be completely satisfactory. First of all, microbial penetration tests were to be performed with films or laminates which have been retorted for 30 minutes at 250°F. Unless some supporting technique was used, all films and laminates curled during the processing. Secondly, if curling was to be eliminated, it was not possible to innoculate or cultivate on the film surfaces under aseptic conditions.

Design of Penetration Test Apparatus

A test device was evolved that met the above requirements and was based on the "surface contact" technique. The test device is shown in Figures 1, 2, and 3 and the blueprint of the device is shown in Figure 4. The entire construction is of Pyrex glass and consists of two identical vessels which are designed to be clamped together. The surface area of the material being exposed to the microbial suspension is 20.58 square inches which was considered the largest and most practical area for a sag-free, unsupported film surface. The injection ports in each half serve two purposes:

1. Equalize the pressure on both sides of the film material during retorting.

2. Allow for the injection or removal of bacterial suspensions in a closed system without disturbing the film.

Test Organisms Used in the Penetration Test Apparatus

In this project, the objective was to study microbial penetration through films into thermally processed foods. In many instances, these foods are classed as non-acid foods and the most critical contaminatinating organism of concern is Clostridium botulinum. This bacteria, however, was not selected for microbial penetration tests since culturing of Clostridia is time-consuming, difficult and not consistently reproducible. Moreover, it was felt that the design of the test equipment and the evaluation procedures involved would become very complex since Clostridia requires special anaerobic conditions for growth.

Since the use of Clostridia was not feasible, two other bacterial species were selected for determining microbial penetration. These were Escherichia coli and Serratia marcescens. These two bacteria resemble Clostridia in several respects, and some of these similarities are summarized in the table below.

Bacteria	Shape	Size (Microns) Diameter	Length	Motility	Optimum Growth Temperature°C
E. coli	rod	0.5-1.0	1.0-3.0	+	30-37°
S. marcescens	rod	0.5	0.5-1.0	+	25-30°
Cl. botulinum	rod	0.5-0.8	3.0-8.0	+	20-30°

Both organisms are easy to grow and maintain in stock cultures, and inoculation cultures can be prepared in less than 16 hours. Although these test organisms are aerobic, they are also capable of becoming facultative anaerobic, an important factor to consider if the organism is to reproduce after penetration.

Use of these two organisms also facilitates the evaluating procedures since the presence of E. coli is widely used as a quantitative and qualitative criterion for evaluating the sanitary aspects of food handling and processing. Many test procedures have been established that can be easily adapted for the purpose of this project. S. marcescens was chosen for its almost spherical shape which may be useful in determining the penetrability of coccoid forms. In addition, the red pigment produced by this organism also provides for rapid detection and identification when penetration occurs.

Penetration Test Procedure of the Individual Films, Aluminum Foils and Laminates in Sheet Form for Microbial Penetration

Sample Preparation:

A minimum of 24 samples of each material were tested for microbial penetration. All film, foils and laminates tested were from roll stock. Samples, approximately one square foot in size, were cut at random from the roll stock. In the center of each sample sheet, a 7.25 inch diameter circle was drawn. The circumference of the circle extends beyond the flange of the glass vessel. The circle constitutes the film surface area which is tested for pinholes and subsequent microbial penetration.

Preliminary Evaluation and Development of Penetration Test Procedure

Two series of preliminary tests were made to check out the reliability of the equipment and to evolve a test procedure. In the first series of tests, two sets of films and laminates were prepared in duplicate containing punctured pinholes of two sizes. Each sample contained four punctured pinholes spaced 1-1/2 inches apart. One set of

materials contained pinholes punctured using a 15 mil diameter needle and another set with a 10 mil needle. In the case of the materials containing pinholes punctured with the 15 mil diameter needle, it was found that both test organisms were able to pass through all the pinholes and reproduce on the agar. From the materials punctured with the 10 mil diameter needle, it was found that with one laminate, 100% microbial penetration did not occur. With the 0.5 mil A Mylar /2.0 mil polypropylene laminate, only 50-75% of the pinholes were penetrated by both test organisms. Examination of the pinholes after the removal of this laminate from the penetration test vessel confirmed that these punctured pinholes were still open.

Using the 10 mil diameter needle, a second series of penetration tests were made on the 0.5 mil A Mylar /2.0 mil polypropylene laminate. Two microbial suspensions of each test organism were prepared. One suspension contained a nonionic wetting agent, the other suspension served as a control. In the penetration tests having the wetting agent included in the microbial suspension, 100% passage was obtained through the punctured pinholes. The wetting agent used was TERGITOL TMN (Union Carbide Corporation). Dilution of the wetting agent to the bacterial suspension was 1:10,000. A dilution of 1:1,000 gave indication of slightly inhibiting bacterial growth in the suspension. (In dilutions above 1:10,000, the effectiveness of the wetting agent was reduced in overcoming the non-wetting properties of the film.)

From these results (using a wetting agent to aid in microbial penetration and to substantiate if wetting agents were a sufficient help), it was decided to use two microbial suspensions of each test organism. One of the bacterial suspensions would contain the wetting agent; the wetting agent was added to the microbial suspension prior to insertion of the suspension into the penetration vessel.

These preliminary tests showed that it was necessary to remove the film material from the penetration test vessel and re-incubate the agar media for 24 hours. Apparently, the removal of the test film resulted in fully aerobic conditions, thereby accelerating growth and permitting easy detection of the penetrating microorganism.

Penetration Test Procedures

Two series of the penetration tests were conducted: the microbial suspension remained in contact with the surface of the material for 72 hours before it was examined for penetration; all the materials were tested in this manner. The second series of tests was made only on the laminates used to fabricate the flexible pouches. In these tests, the microbial suspension was in contact with the material for 30 days. This test procedure was essentially the same as the procedure used for the 72 hour duration test except for the following:

- Serratia marcescens containing the wetting agent was the only microbial suspension used.

- At 72 hour intervals, the bacterial suspension was removed and replaced by a fresh suspension.

- Prior to incubation of the penetration vessel, sterile water was added to that section of the vessel containing the agar media. This sterile water was added to insure that the agar media would remain moist and not dry out.

A minimum of 24 samples of each film, foil and laminate were tested for microbial penetration with the microbial suspension in contact with the material for 72 hours. These samples were tested using the following suspensions:

Number of Samples	Microbial Suspension
6	E. coli
6	E. coli containing the wetting agent
6	S. marcescens
6	S. marcescens containing the wetting agent

The 30 day penetration was conducted using eight samples of each laminate. Time and equipment did not permit a greater number to be tested.

The penetration test procedure was as follows:

1. The ground glass surfaces of the flanges were greased with a silicone vacuum grease. This grease is stable from -40°F to +400°F and is water insoluble (Dow-Corning Corporation).

2. One vessel was placed with the ground flange facing up; 30 ml of liquified Tryptone Glucose Extract Agar media (Difco) was placed into the glass vessel.

3. The film material to be tested is smoothly placed over the up-turned open area of the vessel, including the ground glass surface. (When 2 or 3 ply laminates are used, the side of the film corresponding to the inner surface of a formed pouch is placed face down.)

4. The other half of the glass vessel which serves as a cover, is match-fitted over the bottom vessel and the two halves clamped together to form one unit.

5. The assembled test unit is then placed into a steam retort and is oriented so that the agar media does not make contact with the test material. A 250°F retort temperature is applied for 30 minutes after which time the retort is cooled under pressure.

6. Upon opening the retort, sterile cotton plugs are immediately inserted into the injection ports. The agar media is allowed to cool to approximately 50°C at which time the penetration test vessel is inverted so the agar media will solidify as a thin layer on the surface of the test material.

7. After the agar has solidified, the unit is inverted, placing the solidified agar media on the underside. Twenty ml of an 8 hour suspension (Nutrient Broth, Difco) of one test organisms is injected through the top injection port to cover the surface of the test film. (See Figure 3.)

8. After the incubation period of 72 hours, the bacterial suspension is removed through the injection port, and the cover that contained the bacterial suspension is carefully removed.

10. The test film or laminate is slowly and carefully peeled away from the solid agar in the bottom dish, and replaced with sterile aluminum foil. Following another 24 hours of incubation, the agar surface is examined for microbial growth. The removal of the test film effects a more aerobic condition which accelerates growth and facilitates detection of any microbial penetration of the microorganisms.

11. The film and laminate samples are examined for pinholes regardless if penetration occurred or not.

Abuse Program

The objective in this phase of the project was to study the effect of abusive treatment on the susceptibility of films and laminates to microbial penetration. This program was conducted in two separate phases to determine (a) the effect of creasing on microbial penetration of individual films and laminates in sheet form, and (b) the effect of simulated shipping and rough handling of flexible packages on microbial penetration.

The Effect of Creasing

This study was conducted to determine if creasing is a factor in the microbial penetration of films and laminates. Ten samples of each film and laminate, one square foot each, were creased for this study. The aluminum foils were not tested because of thinness and inherent susceptibility to pinholes. Both test organisms were used with the wetting agent added to the suspension. The creasing procedure was based on the manner described in the Paper Trade Journal, 118, No. 2, TAPPI Section, page 8 (1944). The creasing was done by placing the lightly folded sample under a flat bottom weight. The weight was adjusted to exert a pressure of 6 pounds per inch of crease--e.g., for creasing 12-inch square samples, the creasing load was 72 pounds. Creases were made by gently lowering the weight upon the sample for 15 seconds. Parallel creases were made at 3/4-inch intervals across a given sample. The direction of the creases was alternated to achieve a bellows fold appearance. A second series of creases was then made in the same manner, but at right angles to the first series of creases. After this creasing procedure, the samples were examined for pinholes before the microbial penetration tests.

The Effect of Shipping and Rough Handling

This part of the study conducted was to determine the effect of abusive shipping and handling on filled pouches. Three laminates were used in this study. These laminates are described in detail on page 2. Ninty-six pouches made from each of the three laminates were subjected to shipping and rough handling treatments. The abusive treatments were carried out in sequence. Actually, these treatments were an elimination procedure in which the surviving pouches were tested for microbial penetration. Time and funds did not permit conducting microbial penetration tests on a portion of the pouches after each treatment.

Sealing, Pouch Fabrication, and Fill Material

Prior to fabricating the pouches, it was necessary to conduct a series of investigations to obtain reliable seals. The temperature, dwell period, and pressure was adjusted to give what appeared to be a seal of maximum strength and reliability while at the same time causing the least amount of change to the material adjacent to the seal. The seal tests were conducted in the following manner:

1. Pouches were made containing 150 ml of Nutrient Broth (Difco).

2. The pouches were retorted using the procedure described on page 13

3. After retorting, the pouches were held at room temperature for two hours and then were subjected to 100 pounds of dead weight for 1 minute.

4. Seal strength was determined by cutting three adjacent specimens 1 inch wide, perpendicular to the seal from each edge of the pouch. Specimens were 2 inches in length before opening.

5. Each specimen was opened and one end was attached to a spring scale (maximum reading type) by clamping. The free end was held in a similar clamp and was pulled apart at a uniform rate of 20 inches per minute until failure of the seal or material occurred. Room temperature was within the range of 75±5°F.

The sealing conditions were adjusted until seals would hold even though the film materials failed. Generally, the material failed in the area adjacent to the seal. The laminates containing the polypropylene film failed during the pull test in the following manner: the polypropylene would break adjacent to the seal, followed next by the pulling apart of the aluminum foil and the Mylar. With the laminates containing vinyl, the vinyl film would stretch or pull apart adjacent to the seal, causing a delamination of the aluminum foil and the polyester.

All sealing was done with a resistance heated bar 3/8-inches wide by 12 inches long, producing a seal 3/8-inches wide. The leading horizontal edges of the sealing bar were rounded to eliminate a sharp sealing edge. The striking surface for the sealing bar was an 1/8 inch thick silicone rubber pad. Sealing pressure of the bar was 1.9311 psi. Sealing conditions for the laminates used in the abusive program were as follows:

Laminated Material	Temp. °F	Time (seconds)
0.5 A Mylar /0.35 foil/1.5 polypropylene	380-385	4.0
0.5 A Mylar /0.35 foil/3.0 polypropylene	415-420	4.0
0.5 polyester/0.35 foil/3.0 vinyl	380-385	4.5

Upon obtaining reliable seals, the pouches were fabricated. The material was cut from roll stock in 4-1/2 x 14 inch rectangles. The 4-1/2 inch folded side was sealed. Next, the two 7 inch sides were sealed. The remaining unsealed 4-1/2 inch side was sealed after filling. The completed pouches were 4-1/2 x 7 inches O.D.

Prior to making the final seal, the pouches were filled with 150 ml of Nutrient Broth (Difco). Nutrient Broth is a non-viscous liquid having

a specific gravity of 1.0005. This liquid fill was selected for the following reasons:

- The test organism will grow in the medium very rapidly.
- A liquid will allow immediate detection of pouch failure during abusive treatments.
- The action caused by the movement of non-viscous liquid during the various abusive treatments would be the ultimate in adverse conditions caused by a fill material.
- Nutrient Broth will not plug minute pinholes interfering with the passage of microorganisms.

Retorting

All of the pouches were retorted in a vertical position using a water cook with overriding air pressure. The pouches were placed into a rack with the 4½ inch bottom resting upon a Teflon coated metal screen. The sides of the pouches were supported by aluminum plates 1/16 inches thick, spaced 3/4 or 1 inch apart, depending upon the laminate being retorted. (See Figure Number 10). The rack containing the pouches was placed into the retort containing water preheated to 200°F. Before closing the retort, the water level was adjusted above the rack. The retort was then closed and the automatic pressure gauge set for 27 psi overriding air pressure. The comeup time to 250°F for the water and pouches was approximately 20 minutes. The pouches were held at 250°F for 30 minutes, then were cooled using cold water while maintaining the overriding air pressure. After the pouch temperature had descended below 150°F, the pressure was slowly released.

Packaging the Pouches Before the Abusive Treatments

After removal from the retort, the pouches were air dried. Twenty-four hours after retorting, the pouches were examined for leakers. The pouches were then glued to the center of an overwrap folder (jacket), which was then closed. The folder was constructed of 16 point solid sulfate board conforming to Specification PPP-B-566 (see Figure 6). Folded dimensions were 7-5/8 x 4-5/8 inches. The adhesive used to adhere the pouch to the folder was Resyn 35-6262, manufactured by the National Starch and Chemical Corporation.

Seventy-two pouches enclosed in folders were packed in a fiberboard box in two layers on the edge using a full height partition across the width and a pad between the layers. The partitions produced four sections of 18 pouches each. The fiberboard box was constructed in accordance with Type I, Class 1, style RSC Specification PPP-B-636. The exception was that the fiberboard had a bursting strength of no less than 200 psi

when tested in accordance with method 112 of Specification UU-T-111. The fiberboard cartons were 15.56 x 10.50 x 9.65 inches, stitch box side, B flute, caliper 0.120. The horizontal divider pads were 10.45 x 15.50 x 0.120 inches and the corrugated vertical pads were 4.75 x 10.45 x 0.120 inches. The boxes were securely sealed with three inch wide tape in accordance to class 2 of Specification UU-T-111. The tape extended over all edges of the outer flaps of the box.

The faces, edges and corners of the fiberboard boxes were numbered for identification as prescribed in Standard Method of Drop Test for Shipping Containers ASTM, Designation D 775-61 (see Figure 7). The numbering sequence used to identify the pouch positions within the shipping case is shown in Figure 8. Each shipping container was given a number to identify its contents. The numbering and contents of the containers were as follows:

Box Number	Pouch Material
1	0.5 mil A Mylar /0.35 mil Aluminum foil/1.5 polypropylene
2	0.5 mil A Mylar /0.35 mil Aluminum foil/3.0 mil polypropylene
3	0.5 mil polyester/0.35 mil Aluminum foil/3.0 mil vinyl
4	24 pouches made from each of the above materials to check what effect the abusive treatments would have on pouches of mixed laminates packed together.

The gross weights of the boxes ranged from 28.1 to 28.3 pounds.

Treatments to Simulate Shipping and Rough Handling

The abusive treatment consisted of five separate treatments conducted in sequence; the fifth treatment was designed to induce bacterial penetration. A twenty-four hour hold between each treatment was made to allow for the detection of slow leakers. The abusive treatments were approved by the Project Officer.

Simulated Shipping Treatment

The shipping treatment consisted of vibrating the corrugated carton (containing 72 pouches) for a period of one hour at 1G. The corrugated carton was strapped to a shaker table. (See Figure 12) A .030 inch eccentric cam was used to move the shaking platform up and down in a vertical direction at approximately 1100 rpm. An accelerometer was used to make the final adjustment to 1G. This treatment was used to simulate 3,000 miles of rail or truck transit. After completing this treatment, the corrugated cartons were opened and the pouches examined for material damage and leakage. Leaking pouches were removed and replacements were made bringing the corrugated cartons back up to a fill of 72 jacketed pouches.

Carton Drop Treatment

The corrugated carton containing 72 pouches was dropped in accordance with ASTM D-775-61, Objective B in the prescribed sequence from a height of 18 inches for a total of 30 drops. This treatment was applied to simulate abuse during loading, unloading, stacking, and general handling. After completion of this treatment, the jacketed pouches were removed from the corrugated carton and examined for material damage and leakers. (See Figures 13, 14 and 15).

Jacketed Pouch Drop Treatment

The jacketed pouches were done randomly dropped from a height of 3 feet for 30 drops. This was to simulate handling occurring during the distribution to military personnel and the subsequent carrying of jacketed pouches on person. Each pouch was examined for leakage after each drop and any leakers were removed.

Static Load Treatment

A dead weight of 200 pounds was applied to each jacketed pouch for 3 minutes. The treatment was to insure the integrity of the seals after the previous abusive treatments. After the static load, the pouches were examined for leakers. (See Figure 16.)

Bio-Test Treatment

The "Bio-Testor" is essentially a mechanical device designed to flex one side of the pouch while it is suspended in a water solution containing the test microorganisms. Two metal channels 1-3/4 inches in width and spaced 7/8 inches apart are pressed in an alternating sequence across the $4\frac{1}{2}$ width of the pouch. This kneading action tends to aspirate bacteria, located at a point of micro-leakage into the pouch. This kneading action is provided by two pneumatic tubes resting in the metal channels. The pneumatic tubes are pressurized alternately (line pressure 5 psi) at a rate of 9 seconds per cycle. The Bio-Testor was designed by the Continental Can Company, Inc. in conjunction with their work on Contract No. DA 19-129-AMC-162(N). (See Figures 17, 18 and 19.)

This was the final treatment given to the pouches surviving the Shipping, Carton Drop, Jacketed Pouch Drop and Static Load treatments. The following pouches received the Bio-Test treatment:

- The pouches surviving the Shipping, Carton Drop, Jacketed Pouch Drop, and Static Load treatments.

- Five pouches made of the 3.0 mil vinyl sealant laminate which had been subjected only to the Jacketed Drop and the Static Load treatments.

- Controls:

  a) Five pouches each made of the 3.0 mil vinyl of the 3.0 mil polypropylene sealant laminate. These pouches were only subjected to retorting conditions.

  b) Fifteen pouches each made of the 3.0 mil vinyl and the 3.0 polypropylene sealant laminate. Each pouch contained one pinhole made by puncturing with a 10 mildiameter wire.

The pouches were removed from their fiberboard jackets and placed into the Bio-Testor. The Bio-Testor was then placed into a tank of deionized water containing an 18 hour culture of Serratia marcescens at a population level of 5×10^6 per ml. Serratia marcescens was selected for the following reasons:

- A red pigment is produced by this microorganism under aerobic conditions. The presence of this red pigment in the pouch fill material aids in verifying penetration.

- It is able to reproduce as a facultive anaerobe, which is an important factor to consider if the organism is to reproduce after penetration.

The pouches were flexed for 30 cycles. The pouches were then removed from the Bio-Testor and incubated at 30°C for ten days. This was to allow any organisms that penetrated into the pouch to reproduce in sufficient quantities.

After the ten day incubation period, a 15 ml portion of the fill material was aseptically removed from the pouch and placed into a sterile test tube. This 15 ml portion of fill material is then incubated at 30°C for 3 days. This step was taken to produce an improved aerobic condition to insure red pigment production. The appearance of red pigment signified that microbial penetration (of the test organism) had occurred.

The pouches in which microbial penetration occurred were examined to confirm the presence of pinholes. Each pouch was filled with the crystal violet dye solutions and a slight amount of pressure was applied until the pinhole was detected. This test was to determine whether conditions conducive to microbial penetration developed during the abusive treatments.

III RESULTS AND DISCUSSION

The results obtained in this project are presented in two sections. The first section deals with the microbial penetration studies involving the materials in sheet form. The second section deals with the abusive treatments and microbial penetration of the flexible pouches.

Microbial Penetration of the Materials in Sheet Form

Five hundred and thirty five samples (creased samples included) of the films, aliminum foils and laminates in sheet form were tested for microbial penetration using the penetration test vessels. Microbial penetration occurred in 71 of these tests.

Where microbial penetration occurred, pinholes were found in the material. The growth of the test organism upon the agar media corresponded to the pinhole site on the film, foil, or laminate. The majority of pinholes were found within that area of the material exposed to the surface of the ground glass flange of the penetration test vessel (see Figure 4). Applying vacuum grease to the flange surface reduced pinholes occurring in this area. The exception to this was the aluminum foils, in which the majority of pinholes occurred within the area of the material exposed to the bacterial suspension and the agar media. One test sample each of the 0.35 and 0.70 mil aluminum foil contained two penetration sites, one within the flange area and the other occurring in the center area of the penetration test vessel. In this case, the microbial growth had spread, making it uncertain whether penetration had occurred at both sites. In only one laminate did penetration occur and this occurred in two test samples.

In the 30 day duration penetration test conducted upon the three laminates used for pouch fabrication, penetration did not occur.

The combined testing results of all the individual films, foils, and laminates are listed in Table 1. Table 2 summarizes the location of the penetration sites. The location of the penetration sites occurring in each material are listed and described in Tables 3 through 11. Figure 5 explains penetration sites.

Because no penetration to the test organisms occurred unless a pinhole was present, it was difficult to draw any conclusion from the data as to whether or not the addition of the wetting agent to the microbial suspension aided penetration. In testing the reliability of the penetration test apparatus using pinholes punctured in the films, it was found that the wetting agent was necessary to obtain 100 percent passage of the test organisms. It may be that a naturally occurring pinhole caused by stress or fatigue is entirely different in its configuration from a punctured

pinhole, thus not being as greatly influenced by the non-wetting properties of the films. Because of this difference, the use of a wetting agent may not be necessary in aiding penetration of naturally occurring pinholes.

Problems Encountered With the Aluminum Foil

It was found that minor handling involved in preparing the aluminum foil could cause pinholes. This minor handling consisted of removing the aluminum foil slowly from the stock roll (roll width 13.25 inches), cutting it into 12 inch lengths and conducting the pinhole test. The 0.5 mil foil would pinhole as the result of a slight bend. The 0.35 and 0.70 mil foil appeared to be annealed to a greater extent, thus, enabling it to stand up to abuse during handling to a greater degree than the 0.5 mil foil. Another observation was that the 0.5 foil contained pinholes in it as received, whereas the 0.35 and 0.70 foil were free of pinholes.

In the course of testing the aluminum foils for microbial penetration, it was found that pinholes were occurring in the area of the foil exposed to the bacterial inoculum and the agar media. It was surmised that these pinholes may be the result of conditions experienced during retorting and not the result of chemical action induced by the test microorganisms. In order to confirm our thinking, a short experiment was conducted. Three samples of each film available at the time (0.35 and 0.50 mil), free of pinholes, were placed into the penetration test vessels. The test vessels contained no agar media. The vessels were retorted for 30 minutes at 250°F. All but one film contained pinholes after retorting.

The pinholes were measured by using a B & L microscope with a 10x ocular containing a microscope micrometer disc (B & L 31-16-01) and a 10x objective. Calibration of the ocular micrometer disc was made by placing a micrometer slide (AO 2mm divided into units of 0-.01mm) upon the stage. It was found that each division on the ocular micrometer disc was equal to 7.55 microns.

The pinholes were of the following sizes in microns:

Foil Thickness	0.35 mil	0.50 mil	0.70 mil
Sample No. 1	18.75 x 52.5	7.5 x 7.5	-0-
2	18.75 x 11.25	-0-	-0-
3	37.5 x 15	3.75 x 15	-0-
		41.25 x 45	

Probable Causes of Pinholes in the Films and Laminates

As stated previously, penetration of the test microorganisms did occur, and wherever penetration occurred, pinholes were found in the material. All the materials were examined for the presence of pinholes prior to placement in the penetration test vessels and retorting. Only two samples of the 2.0 mil polypropylene A-22 had pinholes, and these were elminated from the penetration tests. Observation of the films and laminates after

retorting revealed that the following changes in the materials took place, especially with the individual films:

Rippling and Shrinking.
Brittleness.

A number of pinholes was detected within the area of the material making contact with the ground glass flanges. Pinholes were also detected, in a few instances, along the center line of creases in the materials after retorting.

1. Rippling and Shrinking

The area of the film surface subject to contact with the bacterial suspension and agar media rippled in the majority of cases during retorting. This rippling occurred among the following individual films:

1.0 and 0.5 mil Type A Mylar
1.0, 2.0 and 3.0 mil polypropylene A-22.

In the instances where this rippling did not occur among the above listed films, the film was stretched very tightly across the open area of the test vessel. The rippling and stretching* appear to result from the film shrinking. This shrinking and accompanying stresses, it is felt, may be one or a combined factor in producing pinholes.

2. Brittleness

It was observed that the individual films appeared to become brittle or stiff as the result of retort conditions. This was only an observation and no attempt was made to measure these changes quantitatively.

3. Surface Contact with Ground Glass Flanges

The pinhole that occurred in the material coming in contact with the ground glass flange were of the two following types:

Rough edges
Round smooth edges ("fire polished" appearance)

The rough edges appear to be the result of stress; whereas, the smooth edges have the appearance of a hole produced by heat.

In some instances areas of the film and laminates in contact with the flange surface developed a "bubbly" appearance due to retorting. This condition may have resulted because of a combination of pressure produced by the clamps and exposure to heat for a greater time period.

---

*The use of the work "stretching" here means "pulling taut".

4. Creasing of the Material

One crease in the 3.0 mil polypropylene A-22 contained pinholes. This crease occurred on the outer edge of the flange. Another crease having one pinhole was found in the 0.5 mil Type A Mylar /1.5 mil polypropylene A-22 laminate. This crease was examined for pinholes (prior to retorting no pinholes present) and was located in the area exposed to bacterial suspension and the agar media.

Effect of Retorting Upon the Aluminum Foil Interlayer of Laminates

The aluminum foil interlayer of two laminates, 0.5 mil Type A Mylar /0.35 mil aluminum foil/0.15 mil polypropylene A-22 and 0.5 mil polyester/0.35 mil aluminum foil/3.0 mil vinyl, were examined for pinholes using the aluminum pinhole test method described earlier. Existing pinholes were marked. The laminates were then placed into the penetration test vessels and retorted. After retorting the aluminum foil was re-examined for pinholes. It was found that retorting produced pinholes in the aluminum foil of the Mylar /foil/ polypropylene laminate.

Results

Sample No.	No. of Pinholes	Size of Pinholes (Microns)
1	4	(5.5 x 7.5)(5.62 x 7.0)(7.5 x 3.75)(1.9 x 7.5)
2	4	(3.75 x 3.75)(4.2 x 5.6)(5.5 x 3.75)
3	3	(2 x 3.5)(3.75 x 3.75)(1.9 x 7.5)

The area of sample exposed to the retort conditions inside the penetration test vessel was 20.6 square inches per sample.

The pinholes produced were only in the aluminum foil and did not extend through the Mylar or the polypropylene. No penetration occurred through these laminates.

Abusive Treatments

The abusive treatments consisted of two separate programs. One program was to crease the materials in sheet form and the other was to subject pouches to a series of abusive treatments simulating shipping and rough handling.

Creasing Treatment of Individual Sheets

Ten samples of each film and laminate were creased as described in the Experimental Section. No pinholes were found in the materials after creasing.

No microbial penetration occurred along the creases nor were pinholes found along these creases after the penetration test was completed.

Creasing did not bring about microbial penetration in these materials, but this is not to say that creasing would not be a factor leading to microbial penetration. The only other form of abuse to these materials besides creasing was the conditions experienced during retorting. It was reported earlier that retorting appears to impart a brittleness to these materials, especially in the case of the polypropylene. It is therefore possible that flexing these creased materials may produce pinholes enabling microorganisms to pass through.

Pouches Subjected to the Abusive Treatments

A total of 384 pouches (96 of each laminate) were subjected to the five abusive treatments or until failure occurred. An additional 61 pouches were added during the Bio-Test treatment to test the effects of various treatments and to act as controls.

The definition of pouch failure, for this project, is visable leakage of the fill material.

Each pouch was numbered as to its location in the fiberboard carton and as an aid in recording pouch failure (see Figure 7 and 8). Tables 12, 13, 14, and 15 give the details on the type of failure and during what treatment failure occurred for each pouch. Table 16 gives a description of the types of failure for the above four tables. Figure 9 shows graphically three types of failure listed in Tables 12, 13, 14, and 15. Table 17 gives the number of drops for each pouch failing during the Jacketed Pouch Drop Treatment. The following Table A summarizes failures occurring during the abuse test phase of pouches.

TABLE A

SUMMARY OF FAILURES OCCURRING DURING THE ABUSE TEST PHASE OF POUCHES

Type and thickness of sealing surface	Box No. 1	Box No. 2	Box No. 3	Box No. 4 Composite		
	1.5 mil polypropylene	3.0 mil polypropylene	3.0 mil vinyl	1.5 mil polypropylene	3.0 mil polypropylene	3.0 mil vinyl
Number of pouches subjected to Abuse Test	76	72	72	24	24	24
Shipping Test Vibration Test 1 hr.at 1G	4	0	0	0	0	0
Carton Drop Treatment Cartons dropped 30 times from 18"	22	33	10	7**,9***	8**,12***	0
Jacketed Pouch Drop Treatment; Individual pouches dropped 30 times at 36"	43	34	11	9	7	1
Static Load Treatment 200 lbs of pressure on jacketed pouch for 3 min.	7	5	6	5	3	3
Bio-Testor	-	-	4	-	-	-
Total Number of Failures	76*	72	31	31	32	4

Pouch Material:

Box No. 1 0.5 mil A Mylar /0.35 foil/1.5 mil polypropylene
Box No. 2 0.5 mil A Mylar /0.35 foil/3.0 mil polypropylene
Box No. 3 0.5 mil polyester/0.35 foil/3.0 vinyl

*Four pouches were used to replace the pouches that failed during the shipping test.

**Failures resulting from 20 drops, replacements were added to the carton in order to continue the carton drop test.

***Failures resulting from a total of 30 drops or 10 drops in the case of the replacement pouches.

Shipping Treatment

Evidence of material fatigue was present in all the pouches. It was very noticable adjacent to the corners of the pouches. Examination of the aluminum foil interlayer showed that wherever a pouch had a tendency to peak, crease or flex, the foil had slight stress cracks.

Four pouches (Box No. 1) with the 1.5 mil polypropylene sealant material failed. Failure occurred adjacent to the corners.

In the case of the pouches containing the vinyl sealant material, the vibration caused focal points, around which failure of the material occurred in the Carton Drop, Jacketed Pouch, Static Load and Bio-Test treatments.

Carton Drop Treatment

The Carton Drop Treatment inflicted severe damage upon the fiberboard cartons and pouches. Evidence showed at this point that the laminates having polypropylene in their composition could not survive this type of shock. Pouch failures during this treatment were as follows:

30 Drops at 18 inches

Sealant Material	Boxes 1, 2, and 3 (% failures)*	Box No. 4 (% of failures)*
1.5 mil polypropylene	30.6	50
3.0 mil polypropylene	47.2	79.2
3.0 mil vinyl	13.9	0.0

In conducting the Carton Drop Treatment on Box No. 4, the box was dropped initially only 20 times. The liquid fill leaking from the destroyed pouches caused the fiberboard carton to fall apart. A new fiberboard carton plus replacements for the destroyed pouches were used to continue the treatment. It is interesting to note that none of the pouches containing the vinyl failed in Box No. 4.

In the majority of cases, failure of the material occurred on the side that was glued to the fiberboard overwrap (jacket).

The majority of pouches having the polypropylene in the laminate composition failed in this manner: the polypropylene would break adjacent to the seal which in turn brought about the delamination of the aluminum foil and Mylar. The following circumstances may have been responsible for the failure of the polypropylene:

*Failure of replacement pouches was not included in these calculations.

- Retorting causes the polypropylene to become brittle.

- Sealing brings about a change in the polypropylene adjacent to the seal.

- The area adjacent to the seal is the first point of resistance to the movement of the fill liquid caused by the shock due to dropping.

The majority of the polypropylene-laminated pouches surviving the Carton Drop Treatment showed delamination of the aluminum and Mylar in the area of the seal. delamination was caused by the breaking of the polypropylene, the rupture of the foil and Mylar being only a matter of continued abuse.

The pouches made with the laminate containing vinyl failed due to rupture adjacent to the seal or a fatigue induced failure adjacent to the corner seals.

Jacketed Pouch Drop Treatment

The pouches continued to fail in the same manner as described in the results of the Carton Drop Treatment. Table 17 gives the number of drops for each failing during this treatment.

Static Load Treatment

All the pouches made from laminates containing polypropylene surviving the Shipping, Carton Drop and Jacketed Pouch Drop treatments, failed during this treatment. Failure was due to the rupturing of the delaminated aluminum foil and Mylar.

The pouches containing vinyl failed due to the following reasons: 1) rupturing of the material adjacent to the seal, 2) fatigued material failing adjacent to the corner of the pouch, and 3) minute leakage of the fill material caused by the seal opening at the corner of the seal.

Bio-Test Treatment

All microbial penetration occurring due to the Bio-Test treatment was through pinholes in the laminates. The pinholes were caused or detected by the flexing action of the Bio-Testor.

Pouches Surviving the Previous 4 Treatments

Box No. 3: Vinyl Sealant Laminate, 41 pouches treated, 4 pouches failed

Pouch No.	Type of Failure
9	Pinhole adjacent to seal
45	Same as No. 9
47	Fatiguing of Laminate adjacent to corner causing pinhole
48	Same as No. 47

Box No. 4: Vinyl Sealant Laminate, 20 pouches treated, no failures.

Five pouches made of the 3.0 mil vinyl sealant laminate which had been subjected only to the Jacketed Drop and Static Load treatments:
One pouch failed in this group. Failure was due to the delamination of the polyester (outside film) film from the aluminum foil along a vertical crease in the center of the top seal. The test microorganisms traveled along this crease to a pinhole adjacent to the seal.

Controls:

a) Five pouches each made of the 3.0 mil vinyl sealant laminate or the 3.0 mil polypropylene sealant laminate. These pouches were only subjected to retorting conditions. No microbial penetration occurred.

b) Fifteen pouches made of the 3.0 mil vinyl sealant laminate and the 3.0 mil polypropylene sealant laminate. Each pouch contained one pinhole made by puncturing with a 10 mil diameter wire. All these pouches experienced microbial penetration.

Abusive Treatment Aftermath Study

Reviewing the number of failures resulting from the Carton Drop Treatment and the damage inflicted upon the surviving pouches, a short test was conducted to determine if the Carton Drop Treatment was the major contributing factor in pouch failure. Eight pouches made from each laminate were treated in the following manner:

1. Three pouches of each laminate were subjected to Static Load Treatment. No failures occurred.

2. The remaining five pouches were subjected in sequence to Jacketed Drop Treatment and the Static Load Treatment. The results were as follows:

Laminate - Sealant Material	Jacketed Pouch Drop Treatment	Static Load Treatment	Pouches Remaining
1.5 mil polypropylene	1 @ 20 drops	4	0
3.0 mil polypropylene	1 @ 28 drops		
	1 @ 30 drops	3	0
3.0 mil vinyl	-0-	0	5

The above data indicate that 30 drops from 36 inches (Jacketed Pouch Drop Treatment) caused sufficient damage to the laminates, containing the polypropylene, that these pouches could not survive the subsequent Static Load Treatment. All failures were due to the polypropylene material breaking adjacent to the seal.

Problems Encountered in Retorting Pouches

The pouches were retorted as previouslv described in the Experimental Section (Section II). The pouch support plates of the holding rack were spaced 3/4 or 1 inch apart. The 0.5 A Mylar /0.35 foil/1.5 polypropylene and the 0.5 polyester/0.35 foil/3.0 vinyl laminates were retorted with the support plates being 3/4 of an inch apart. When retorting the 0.5 A Mylar /0.35 foil/ 3.0 polypropylene laminate with the support plates being 3/4 of an inch apart, it was found that a blister type of delamination occurred separating the A Mylar from the foil. These blisters were from 1 to 2 mm in diameter and occurring within the area of the film coming in contact with the support plates. It was surmised that localized calcium deposites on the support plates combined with the laminate being exerted against the plate may be one factor in causing this delamination. An experiment was made using new and clean aluminum plates and enclosing half of the pouches in an envelope of 0.5 A Mylar. These changes in conditions did not remedy this delamination problem. It was found that by spacing the support plates 1 inch apart, this delamination did not occur.

IV. RECOMMENDATIONS

The following recommendations are offered for consideration by investigators in future work related to microbial penetration of flexible packaging materials:

A. For studies related to determining the smallest size pinhole a microbial agent of a specific size will penetrate, the following suggestions are offered.

   1. Select microorganisms having both the form of rod and cocci (spherical) to determine if the shape of the organism has an effect on its ability to pass through a pinhole.

   2. Select both motile and non-motile microorganisms to determine whether this characteristic is important in obtaining penetration.

   3. Determine the sensitivity and limitations of the pinhole test methods selected for the study.

   4. Develop a reproducible method for making pinholes of various sizes in the range of 0.5 microns to 100 microns. Electric discharge and laser techniques are suggested as possible methods.

   5. Study the physical characteristics which can encourage penetration such as flexing, pressure changes, and presence of wetting agents.

B. Study the abuse resistance of flexible packages with various types of contents. The following recommendations are offered.

   1. Plan to prepare and test several thousand pouches.

   2. Fill pouches with liquids of several viscosities, solids of several densities and particle sizes and solid-liquid mixtures.

   3. Test a portion of the pouches for microbial penetration after each abuse treatment and the remainder after the full series of abuse treatments or gas penetration detection.

C. Study methods for high speed detection of pinholes in roll film material using new techniques of optical scanning.

V. REFERENCES

1. Hartman, M. W. "Procedure for Measuring Bacterial Permeability of Food Packaging Films." M. S. Thesis, University of Georgia Library, Athens, Georgia. 1962

2. Proctor, B. E. and J. T. R. Nickerson, "Investigation of Bacterial Resistance of Packages." Report of QM Research Contract DA 19-129-AM758, MIT, Cambridge, Massachusetts. 1958

3. LeFebvre, P. H., "Une Nouvelle Methode Rapide de Detection et de Localisation des Fuites Microscopiques des Boites a Conserves. Revue des Fermantations et des Industries Alimentaires, 5, 183-188, 1950

VI. APPENDIX

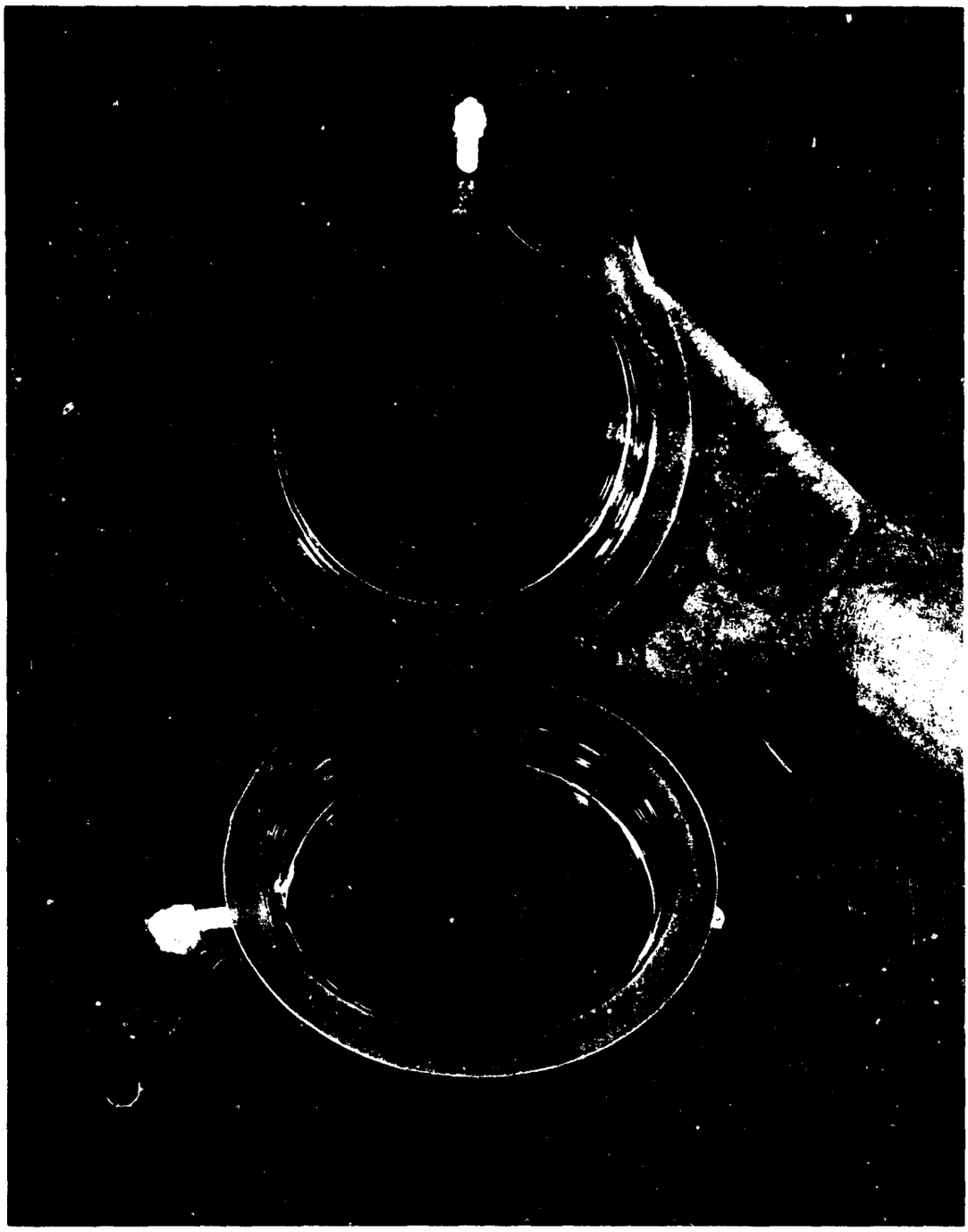


FIGURE 1 BACTERIAL PENETRATION TEST APPARATUS UNASSEMBLED

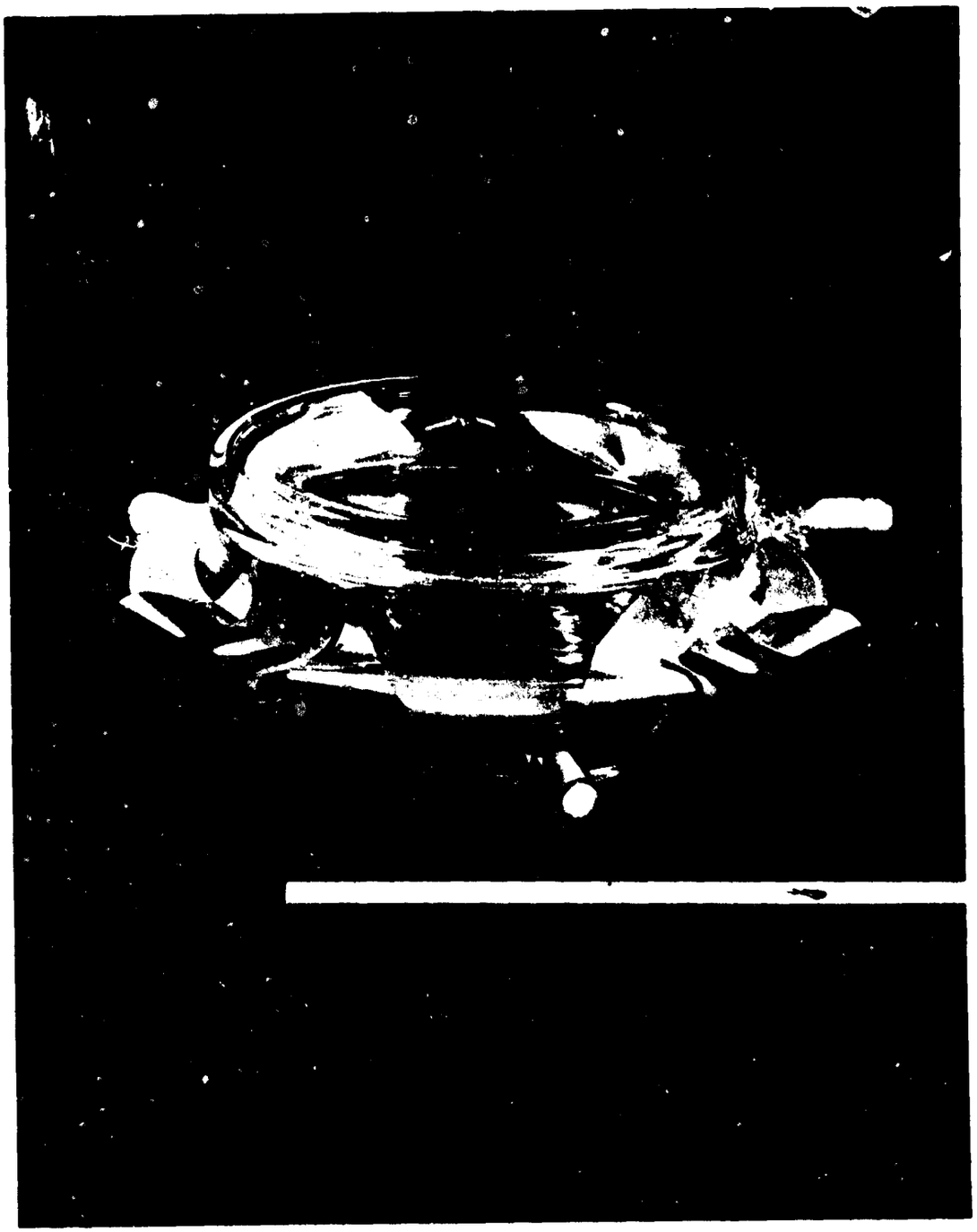
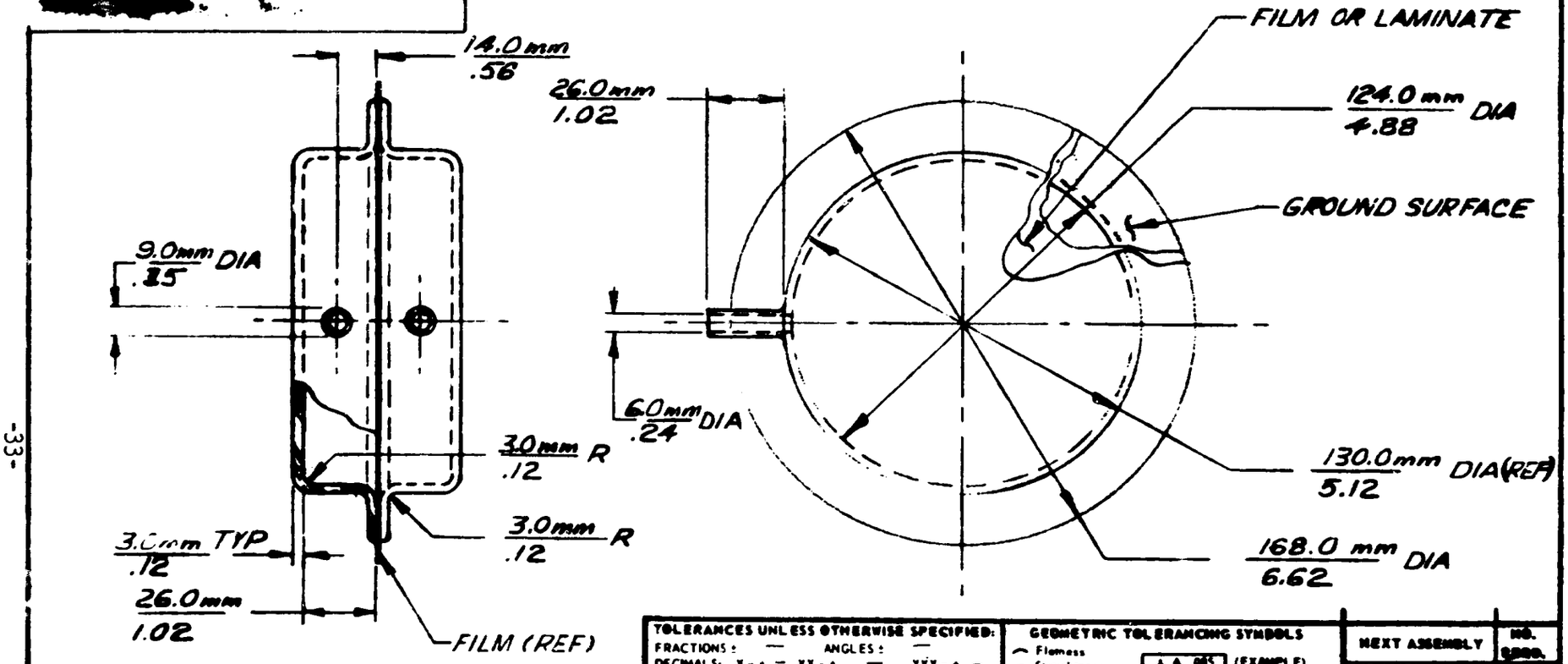


FIGURE 2 BACTERIAL PENETRATION TEST APPARATUS ASSEMBLED

FIGURE 3 INJECTION OF MICROBIAL TEST ORGANISM INTO PENETRATION TEST VESSEL

[redacted]

Fig. 4



TOLERANCES UNLESS OTHERWISE SPECIFIED:
FRACTIONS ± — ANGLES ± —
DECIMALS: .X = ± — .XX = ± — .XXX = ± —

Break Sharp Edges .005-.015 R, or Chamfer
Surface Roughness Symbol, Ref. ASA 46.1
Welding Symbols, Ref. ASA Y32.3
Electric Symbols, Ref. ASA Y32.2
Hydraulic Symbols, Ref. ASA 32.10

GEOMETRIC TOLERANCING SYMBOLS
Flatness
Straightness
Angularity
Perpendicularity
Parallelism
Concentricity
True Position

⊥ A .005 (EXAMPLE)
Tolerance
Reference Plane or Axis Symbol

INTERPRETATION:
Plane Indicated is Perpendicular to Plane A Within .005

NEXT ASSEMBLY | NO. USED ON
FIRST MADE FOR

FMC CORPORATION
CENTRAL ENGINEERING LABORATORIES

SYM.	E.O.	REVISIONS	BY	CH.	APP.	DATE

	BY	DATE
DR.	JRO	11/1/65
CH.		
APP.	[redacted]	
APP.		
SCALE	1/2	

PENETRATION TEST APPARATUS

MATERIAL: PYREX GLASS

DWG. SIZE: A

DWG. & PART NO.

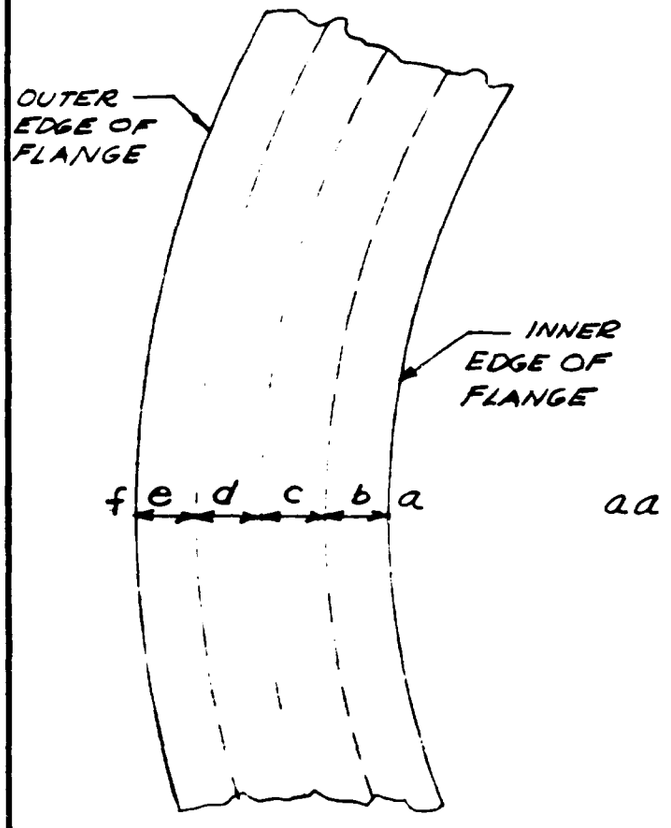


Figure 5

Sites of pinholes occurring in the materials tested for penetration in relation to the ground glass flanges of the test vessels.

aa	includes center area up to 1/8" of flange.
a	extends from inner edge of flange toward center of vessel 1/8".
b,c,d, and e	are 3/16" increments reading from the inner edge of flange to outer edge.
f	extends from the outer edge of the flange and away from the flange 1/8".

Outer diameter of test vessel is 7.25 inches.

TOLERANCES UNLESS OTHERWISE SPECIFIED:
FRACTIONS ± ANGLES ±
DECIMALS X ± XX ± XXX ±
Break Sharp Edges .005/.015 R. or Chamfer
Surface Roughness Symbol, Ref. ASA 46.1
Welding Symbols, Ref. ASA Y32.3
Electric Symbols, Ref. ASA Y32.2
Hydraulic Symbols, Ref. ASA 32.10

GEOMETRIC TOLERANCING SYMBOLS
Flatness
Straightness
Angularity
Perpendicularity
Parallelism
Concentricity
True Position
⊥ A .005 (EXAMPLE)
Tolerance
Reference Plane or Axis
Symbol
INTERPRETATION
Plane Indicated is Perpendicular to Plane A Within .005

NEXT ASSEMBLY | NO. REQD
FIRST MADE FOR

FMC CORPORATION
CENTRAL ENGINEERING LABORATORIES
1618 Orrington Avenue Evanston

SYM	E C N or E O	REVISIONS	BY	CK	APPD	DATE

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DR	FHJ	3/10/66
CHK		
APPD		
APPD		
SCALE	TWICE	–

DIAGRAM - FLANGE RANGES
PENTRATION TEST APPARATUS

MATERIAL | DWG SIZE: A | DWG & PART NO: Fig. 5

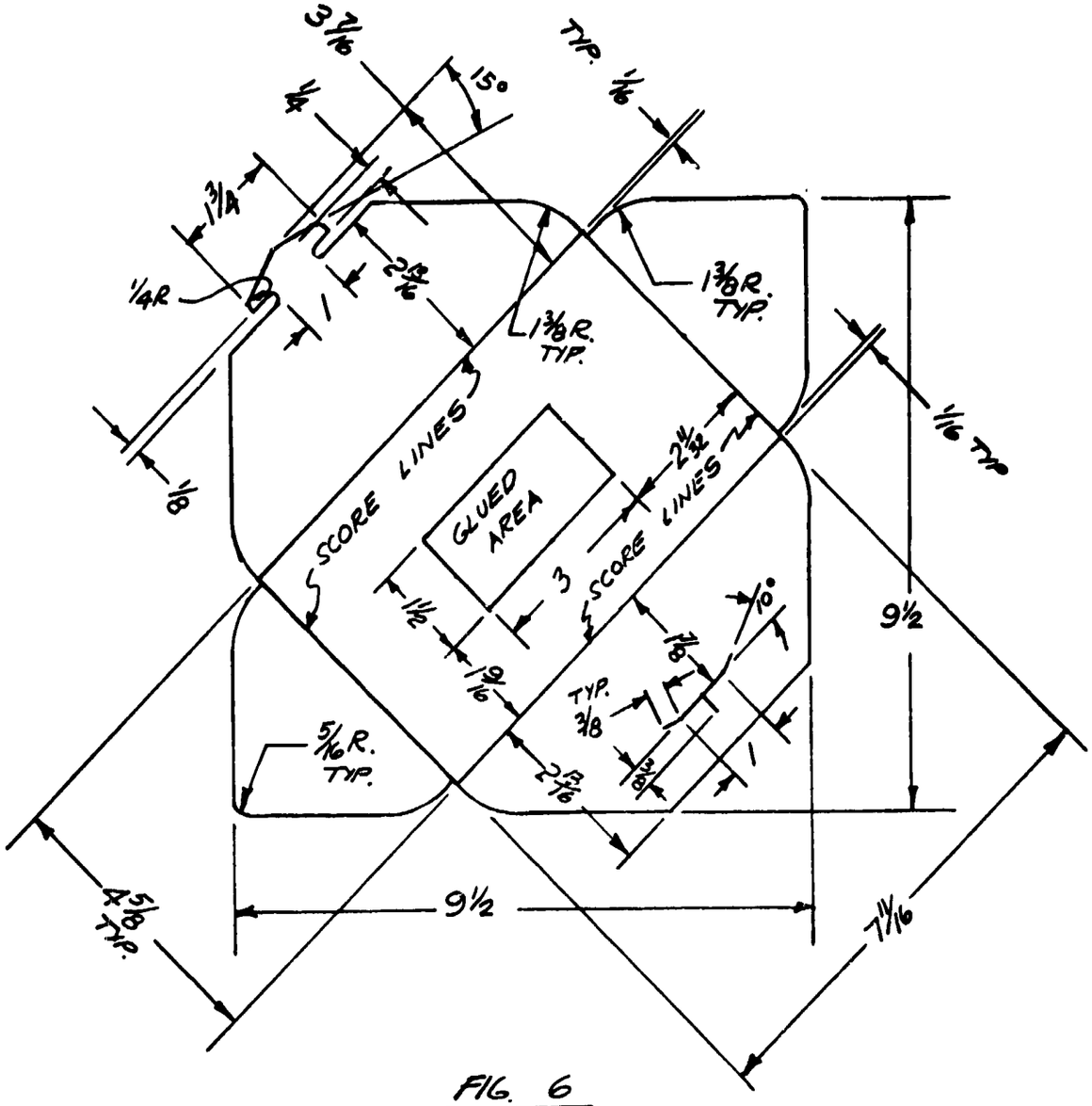


FIG. 6
FOLDER BLANK

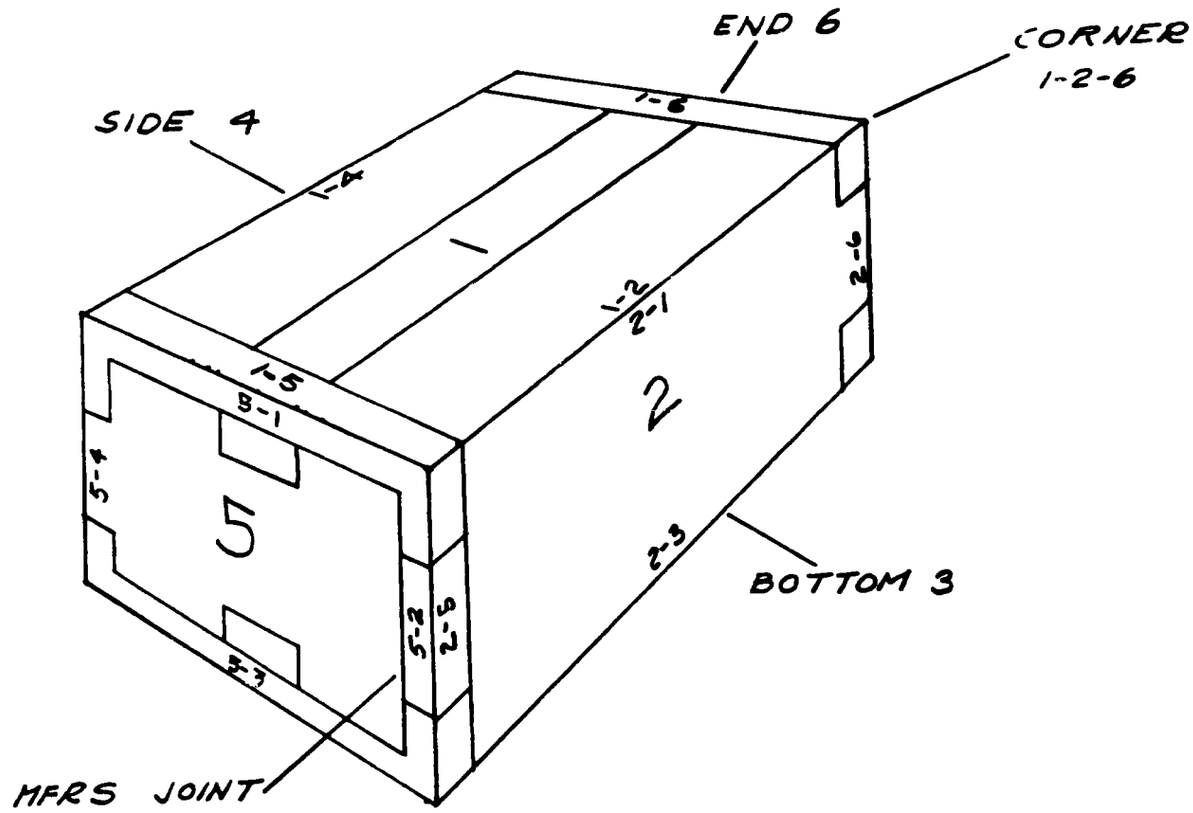
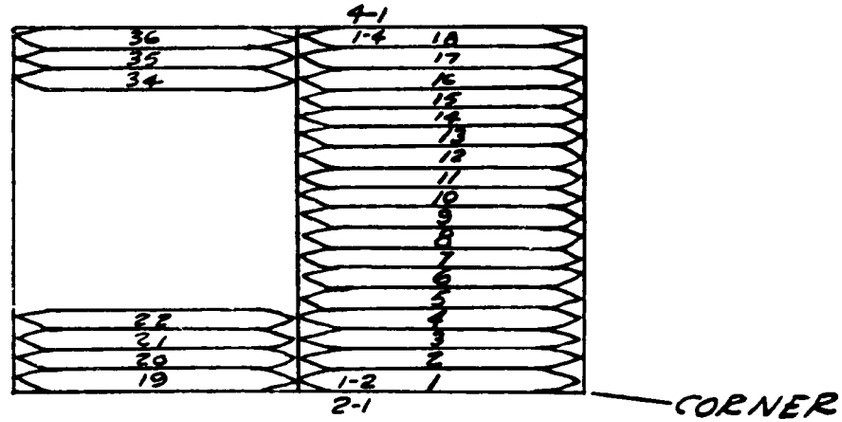
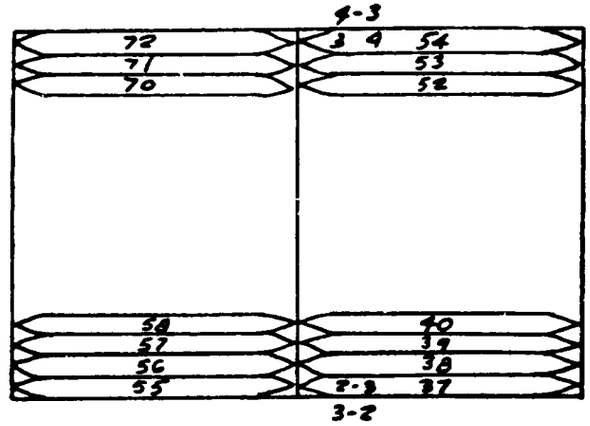


FIG. NO 7

FIBERBOARD CARTON NUMBERING SYSTEM



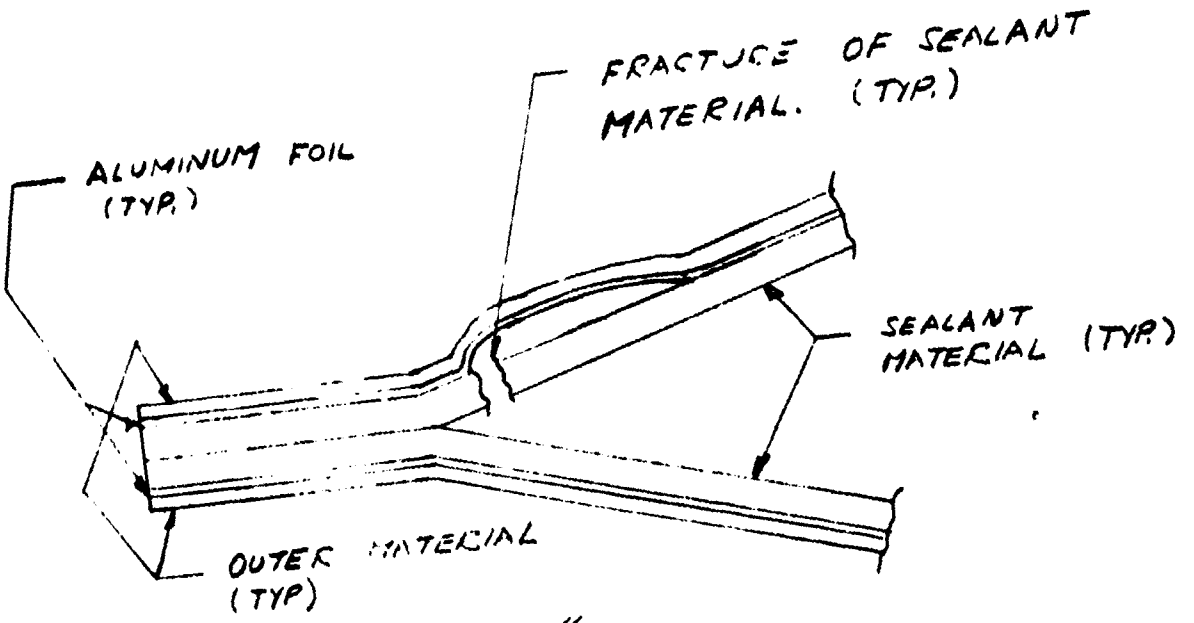
TOP LAYER



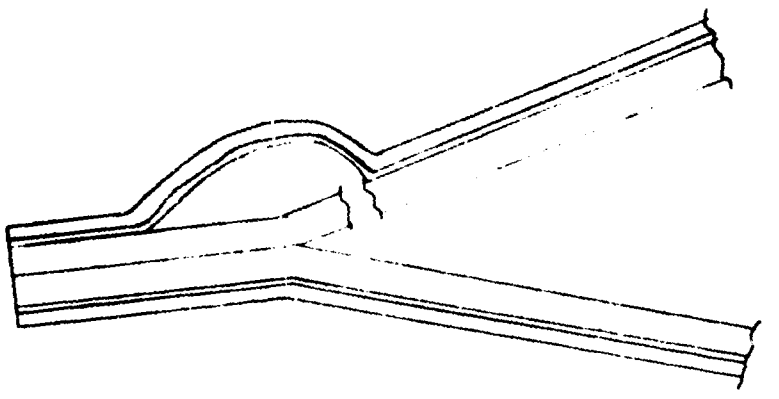
BOTTOM LAYER

FIG. NO. 8

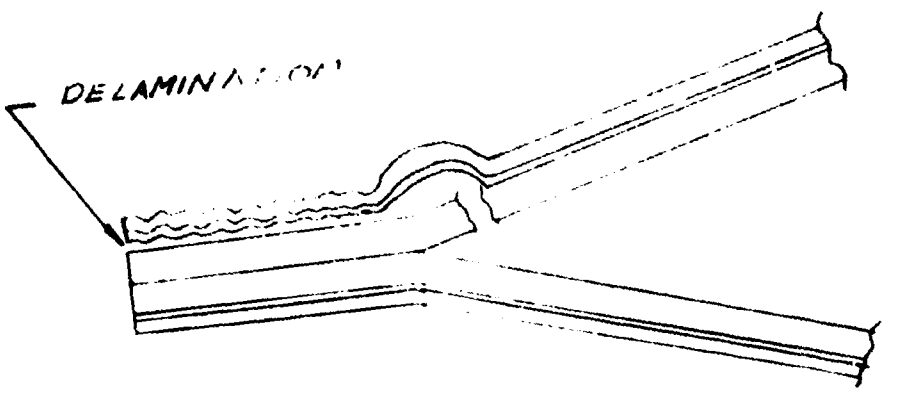
POUCH POSITION IN FIBERBOARD CARTON



TYPE "B"



TYPE "C"



TYPE "E"

FIG. NO 9

THREE TYPES OF MATERIAL FAILURE

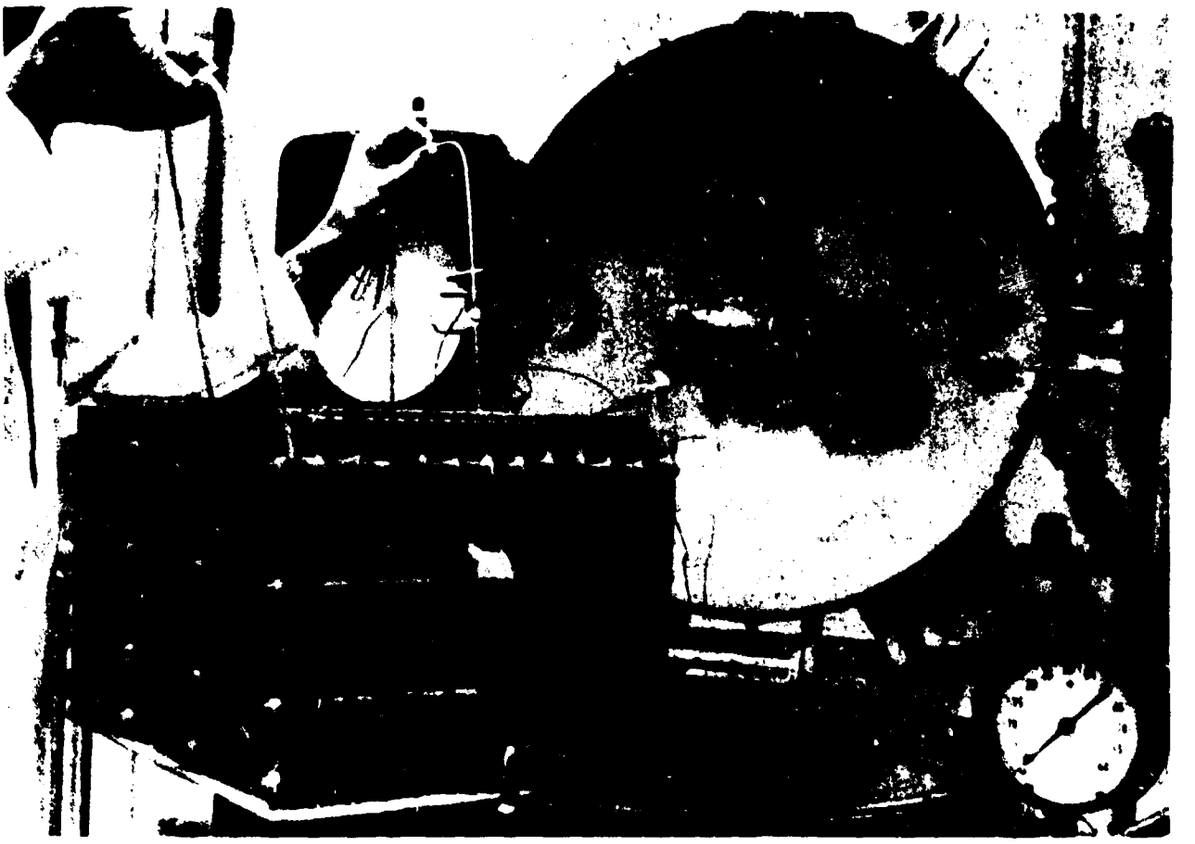


FIGURE 10 POUCH SUPPORT RACK USED FOR RETORTING

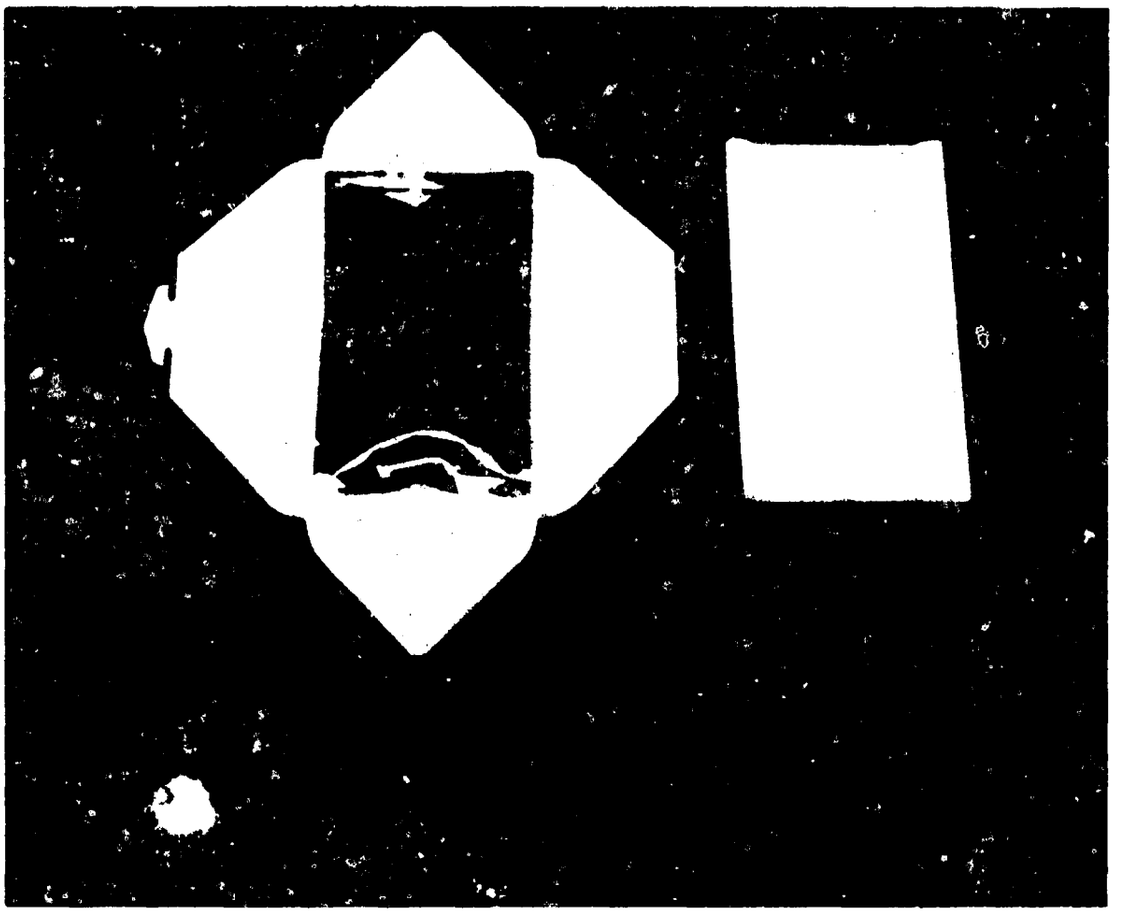


FIGURE 11 OPEN AND CLOSED FIBERBOARD JACKET

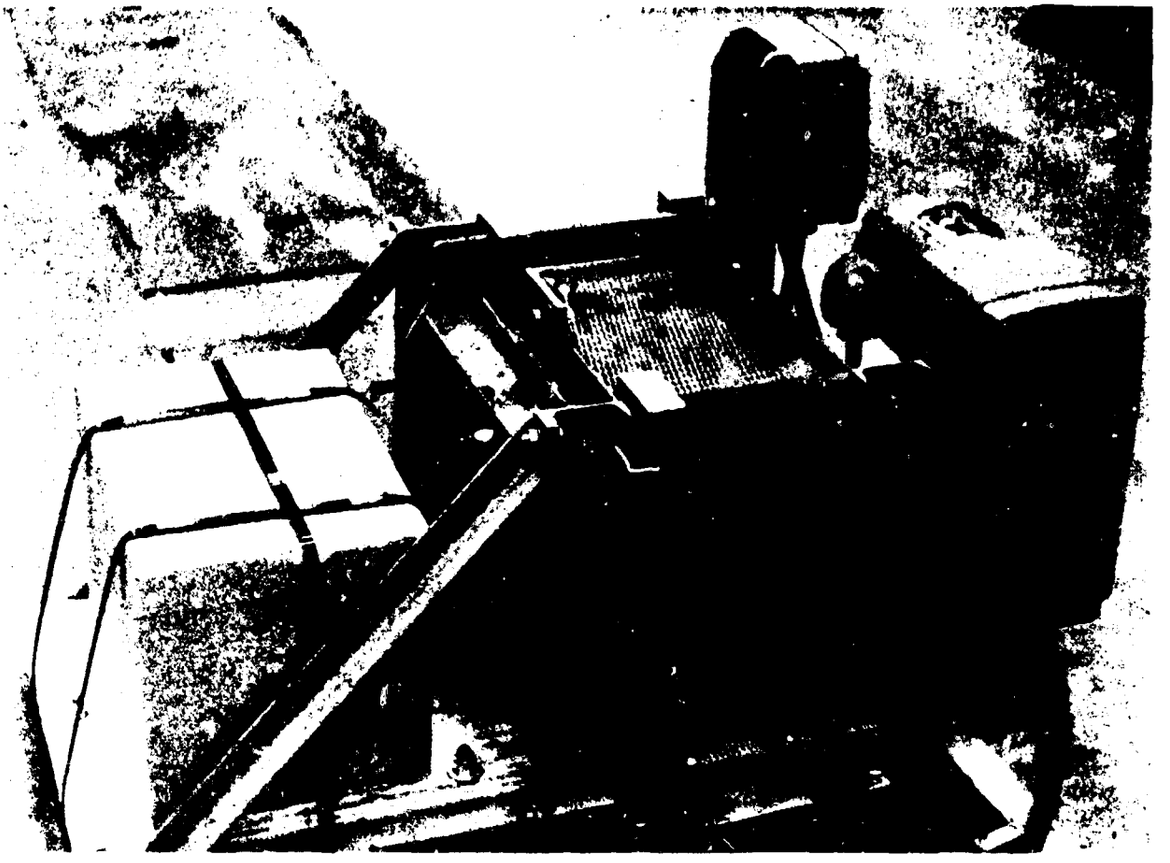


FIGURE 12 SHAKER TABLE USED FOR THE SHIPPING TREATMENT

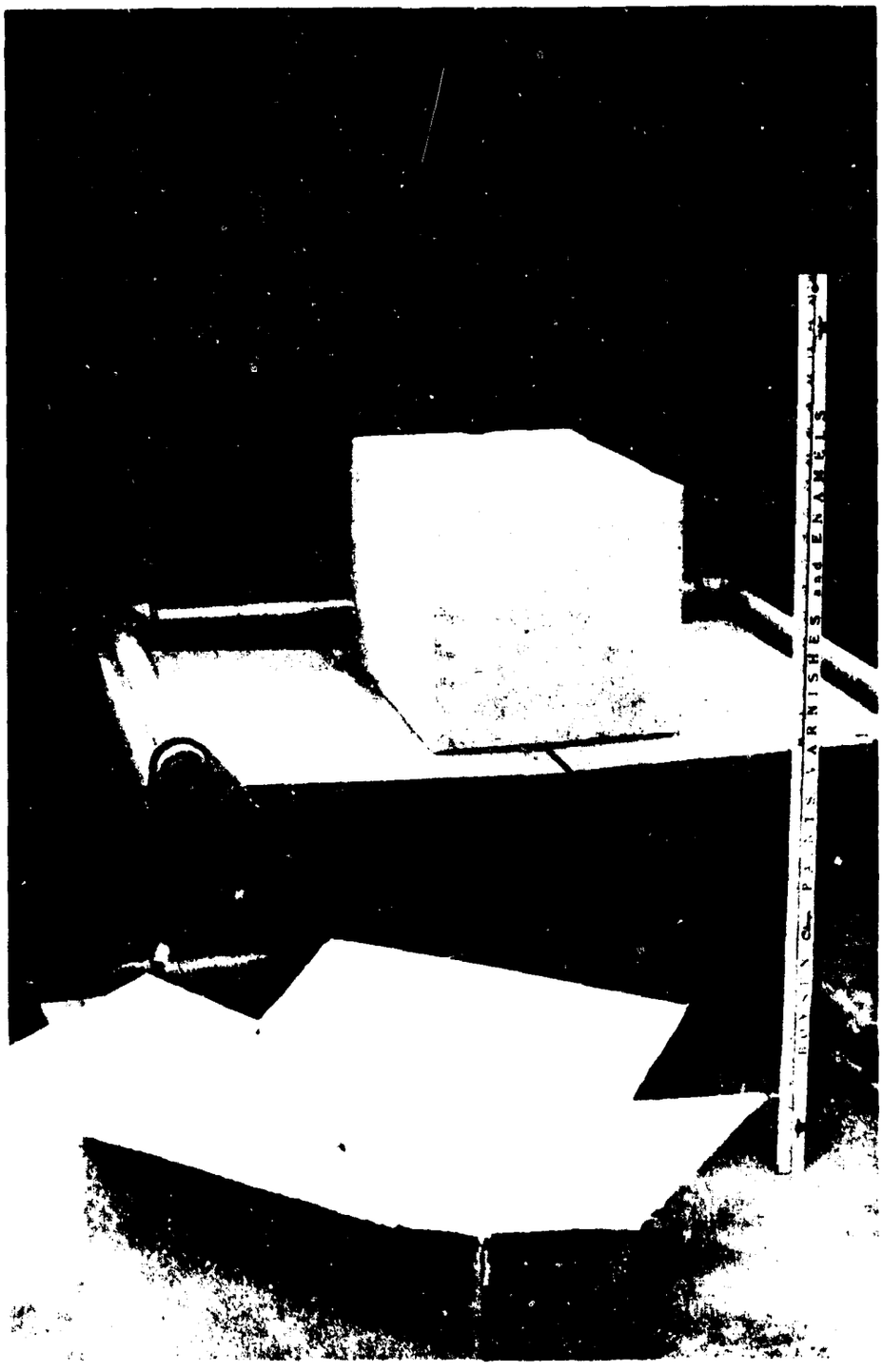


FIGURE 13 CARTON DROP TREATMENT

FIGURE 14 BOX NO. 3 AFTER CARTON DROP TREATMENT

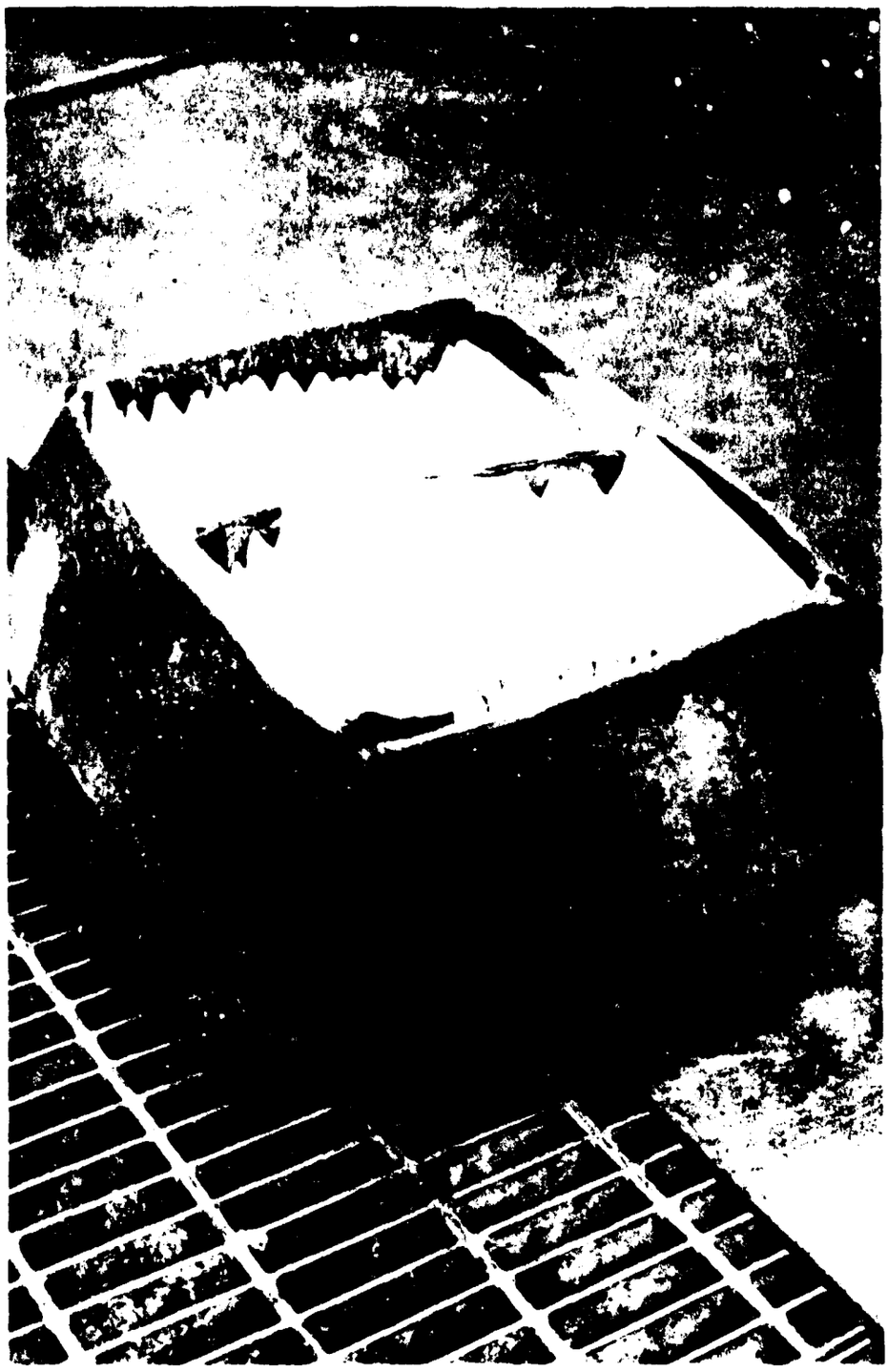


FIGURE 15 BOX NO. 4 AFTER CARTON DROP TREATMENT

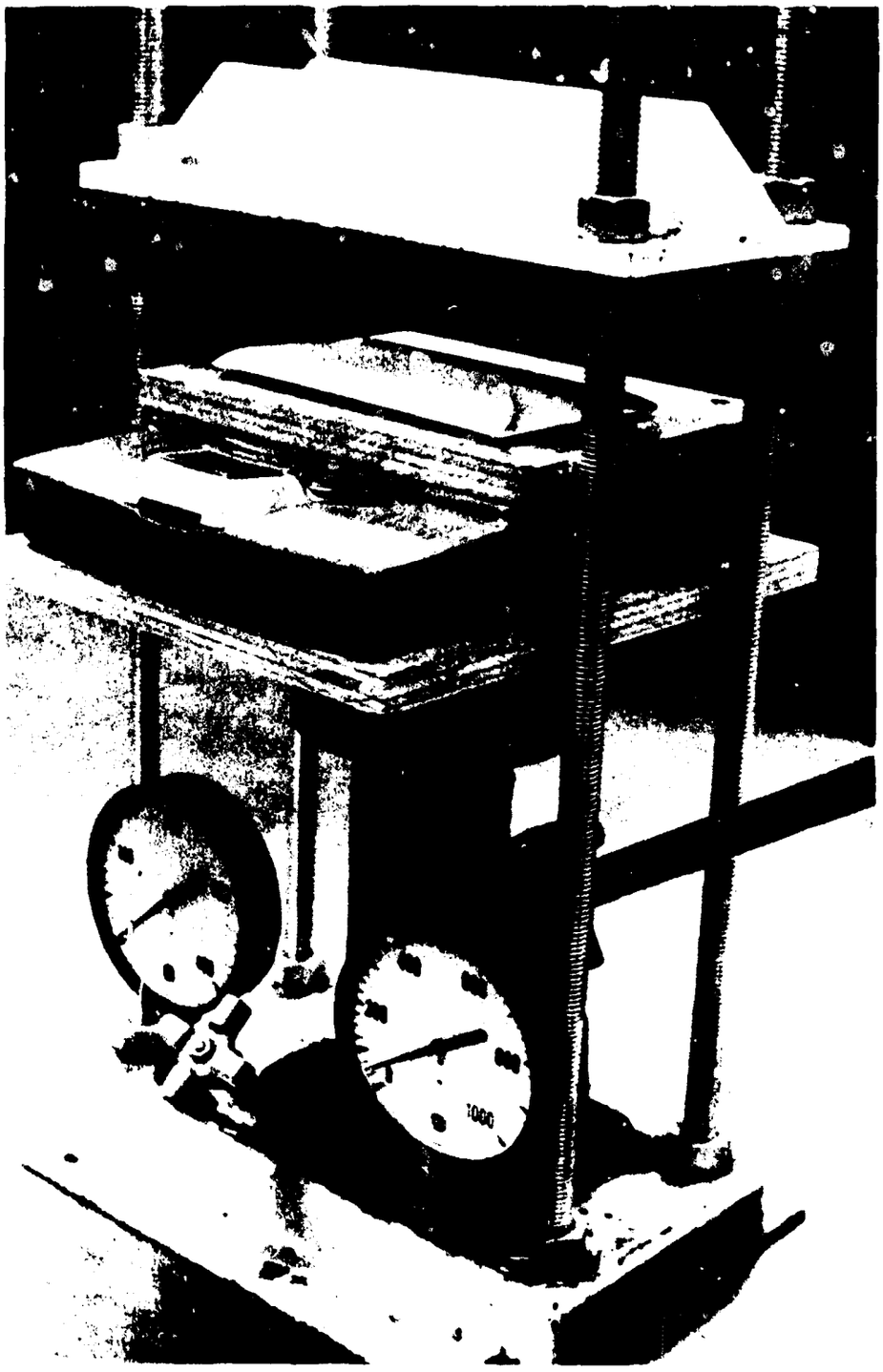


FIGURE 16 STATIC LOAD TREATMENT

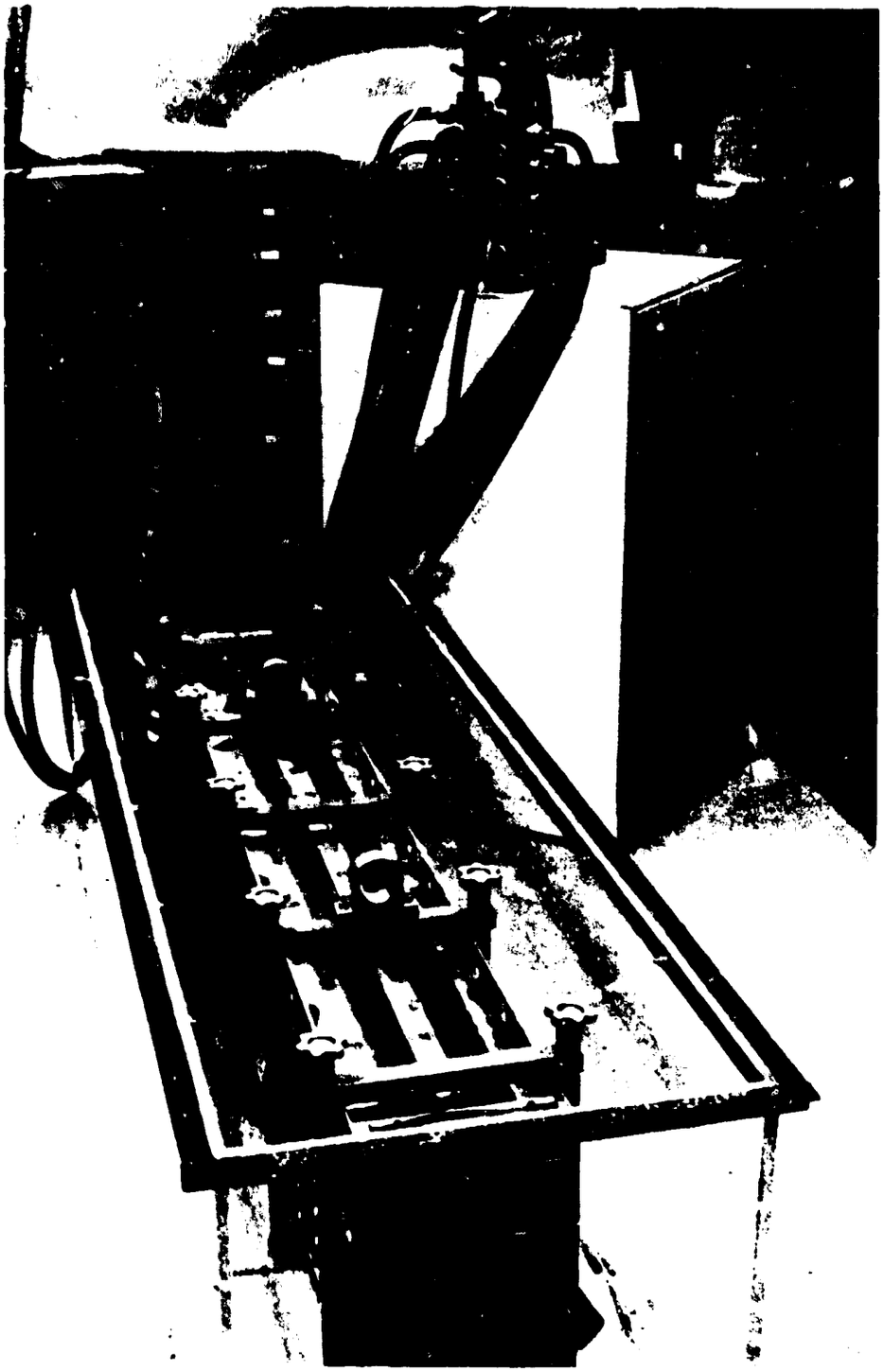


FIGURE 17 BIO-TESTOR

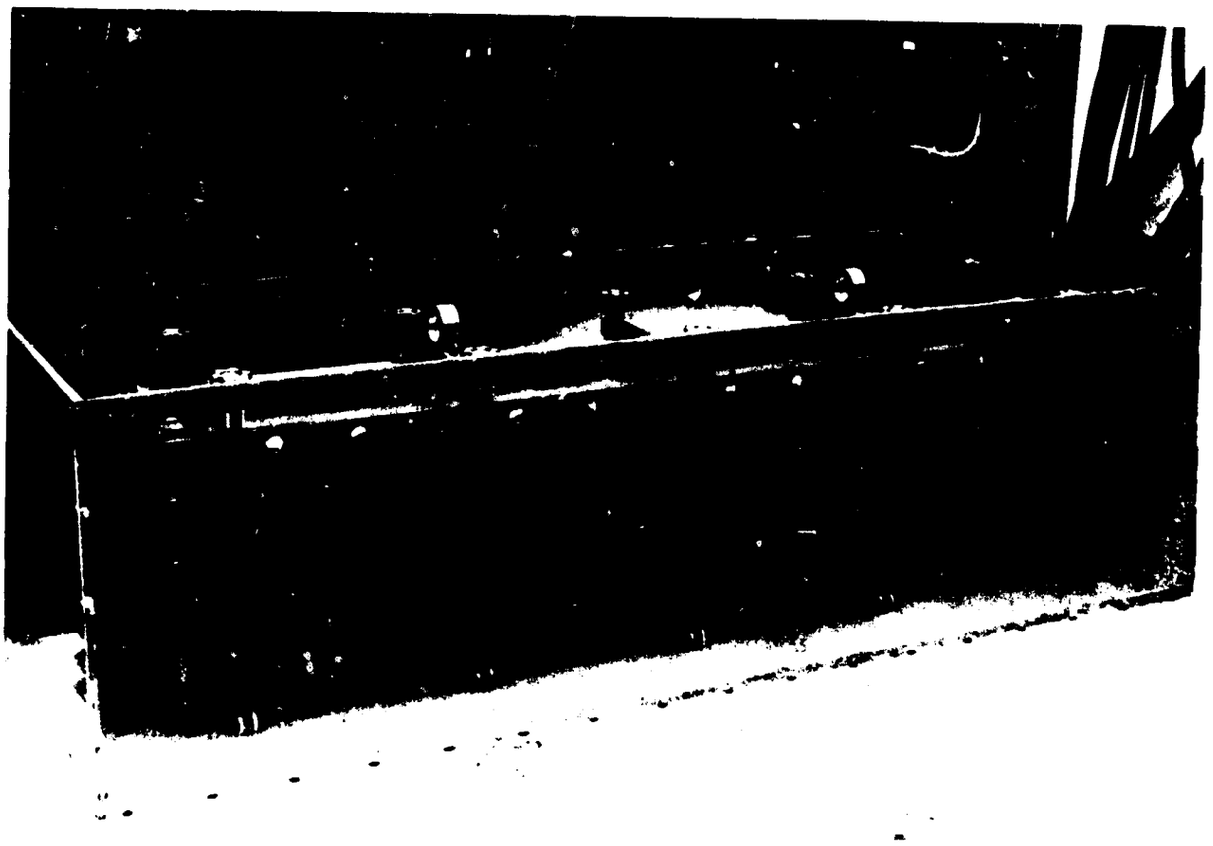


FIGURE 18 BIO-TESTOR

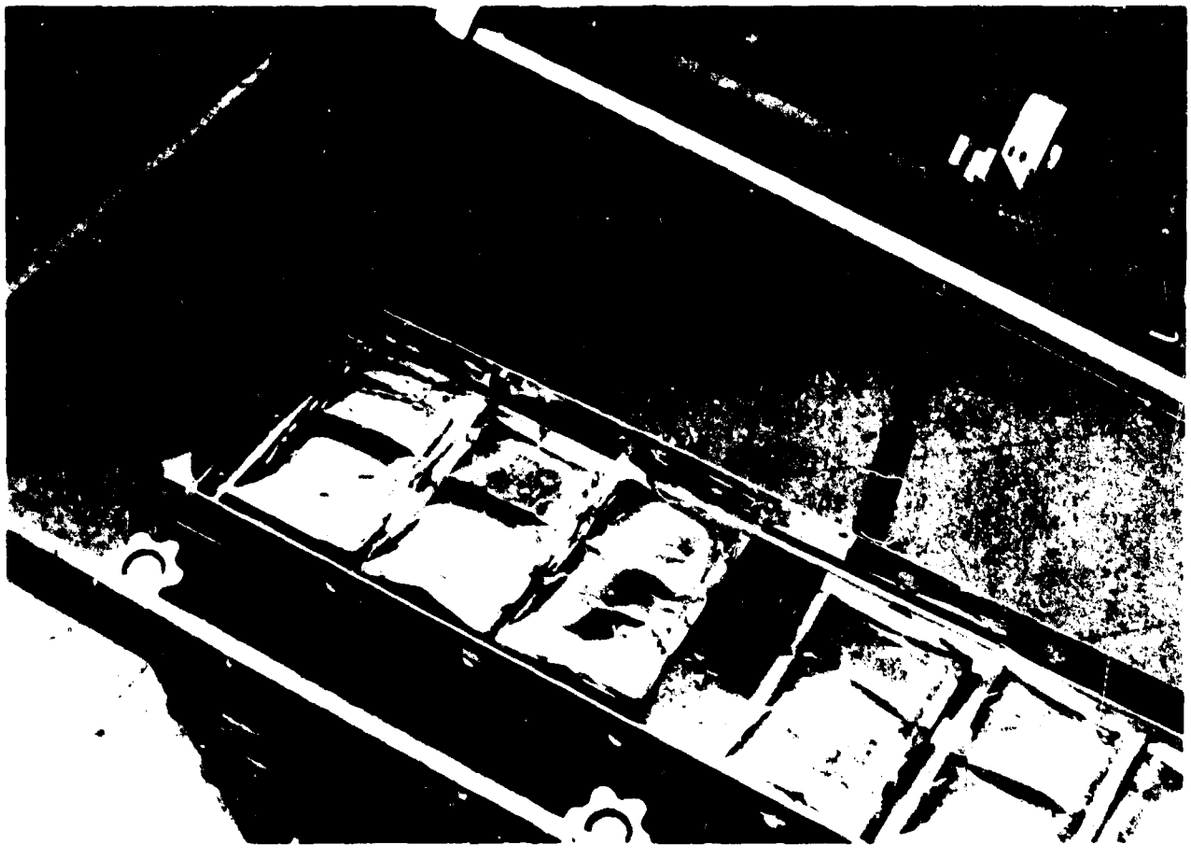


FIGURE 19 POUCHES IN BIO-TESTOR PRIOR TO REMOVAL AFTER FLEXING

Table 1

NUMBER OF MICROBIAL PENETRATIONS FOR MATERIAL TESTED

Test Organisms	E. coli				S. marcescens					
Wetting Agent	w/wetting agent				w/wetting agent				Total Samples	REMARKS
Penetration	yes	no	yes	no	yes	no	yes	no		Penetration Causes
Description material tested*										
Individual Films:										
0.5 "A Mylar before greasing flange.	4	3	5	2	5	2	3	4	28	Pinholes produced in flange area; materials tore on removal from penetration vessels.
0.5 "A Mylar" after greasing flange	0	5	2	3	0	5	1	4	20	Pinholes produced in flange area.
0.75 "A Mylar"	0	6	0	6	0	6	0	6	24	
1.0 "A Mylar"	0	7	0	7	0	7	0	7	28	
0.35 Aluminum Foil	2	4	3	3	1	4	4	2	23	Pinholes produced in area of material expose to test organism and flange area.
0.5 Aluminum Foil	4	4	8	1	5	3	6	1	32	" " " " "
0.7 Aluminum Foil	0	7	0	7	1	6	3	4	28	" " " " "
1.0 Polypropylene A-22	0	6	1	5	2	4	1	5	24	Pinholes produced in flange area.
2.0 Polypropylene A-22	0	7	0	5	1	6	0	5	24	
3.0 Polypropylene A-30	1	5	0	7	0	6	1	5	25	
Laminates										
0.5 "A Mylar"/1.5 polypropylene A-22	0	6	1	5	1	5	0	6	24	Pinholes produced in area of material expose to test organism and flange area.
0.5 "A Mylar"/2.0 polypropylene A-22	0	6	0	6	0	6	0	6	24	
0.5 "A Mylar"/3.0 polypropylene A-30	0	6	0	6	0	6	0	6	24	
0.5 "A Mylar"/0.35 foil/ 1.5 polypropylene	0	6	0	6	0	6	0	6	32	
0.5 "A Mylar"/0.35 foil/ 3.0 polypropylene	0	6	0	6	0	6	0	6	32	
0.5 polyester/0.35 foil/ 3.0 vinyl	0	6	0	6	0	6	0	6	32	

*All material thickness in mils (0.001)

Table 2

Summary of Location of Penetration Sites

Individual Films	Flange Surface	Center Area of Penetration Test Vessel
E. Coli	4	0
E. Coli - W. A.	7	2
S. Marcescens	8	0
S. Marcescens - W. A.	5	1
Aluminum Foil		
E. Coli	0	6
E. Coli - W. A.	3	8
S. Marcescens	2	7
S. Marcescens - W. A.	4	12
Laminates		
E. Coli	0	0
E. Coli - W. A.	1	0
S. Marcescens	0	1
S. Marcescens - W. A.	0	0

Table 3

DISTRIBUTION OF PENETRATION SITES

Material:

0.5 mil A mylar
Before greasing flanges

Test Microorganism		Total No. of positive samples	Positive Replicate No.	Distribution of Penetration Sites							Total sites per replicate
				aa*	a	b	c	d	e	f	
E. coli	without wetting agent	4	1			1					1
			2					1**			1
			3							2**	2
			4				1				1
E. coli	with wetting agent	5	1					1			1
			2		1						1
			3							1**	1
			4							1**	1
			5							1**	1
S. marcescens	without wetting agent	5	1				1				1
			2							1	1
			3				1				1
			4							1	1
			5							1	1
S. marcescens	with wetting agent	3	1				1				1
			2			1					1
			3						1		1

** (tears)

* See Fig. 3 for penetration site code

DISTRIBUTION OF PENETRATION SITES

Material:

0.5 mil "A Mylar"
after greasing flanges.

Test Microorganism		Total No. of positive samples	Positive Replicate No.	Distribution of Penetration Sites							Total Sites
				aa*	a	b	c	d	e	f	
E. coli	without wetting agent	0									
E. coli	with wetting agent	2	1 2		1				 1		1 1
S. marcescens	without wetting agent	0									
S. marcescens	with wetting agent	1	1							1	1

* See Fig. 3 for penetration site code

TABLE 5

DISTRIBUTION OF PENETRATION SITES

Material:

0.35 mil Aluminum Foil

Test Microorganism		Total No. of positive samples	Positive Replicate No.	Distribution of Penetration Sites							Total Sites
				aa*	a	b	c	d	e	f	
E. coli	without wetting agent	2	1	1							1
			2	1							1
E. coli	with wetting agent	3	1		1						1
			2	1							1
			3	1							1
S. marcescens	without wetting agent	1	1	1			1				2
S. marcescens	with wetting agent	4	1	1							1
			2	1							1
			3	1							1
			4					1			1

* See Fig. 3 for penetration site code

Table 6

DISTRIBUTION OF PENETRATION SITES

Material:

0.5 mil Aluminum Foil

Test Microorganism		Total No. of positive samples	Positive Replicate No.	Distribution of Penetration Sites							Total Sites
				aa*	a	b	c	d	e	f	
E. coli	without wetting agent	4	1	1							1
			2	1							1
			3	1							1
			4	1							1
E. coli	with wetting agent	8	1							1	1
			2	1							1
			3		1						1
			4					1			1
			5	1							1
			6				1				1
			7	1							1
			8	1							1
S. marcescens	without wetting agent	5	1	1							1
			2	1							1
			3	1							1
			4	1							1
			5	1							1
S. marcescens	with wetting agent	6	1	1							1
			2	1							1
			3	1							1
			4	1							1
			5	1							1
			6	1							1

* See Fig. 3 for penetration site code

Table 7

Material: DISTRIBUTION OF PENETRATION SITES

7.0 mil Aluminum Foil

Test Microorganism		Total No. of positive samples	Positive Replicate No.	Distribution of Penetration Sites							Total Sites
				aa*	a	b	c	d	e	f	
E. Coli	Without wetting agent	0	0								0
E. Coli	With wetting agent	0									0
S. marcescens	Without wetting agent	1	1 2		1	 1					2
S. marcescens	With wetting agent	3	1 2 3		1 1 1	1 1 1					6

*See Figure 3 for penetration site code

DISTRIBUTION OF PENETRATION SITES

Material:

0.0 mil Polypropylene A-22

Test Microorganism		Total No. of positive samples	Positive Replicate No.	Distribution of Penetration Sites							Total Sites
				aa*	a	b	c	d	e	f	
E. coli	without wetting agent	0									0
E. coli	with wetting agent	1	1							1	1
S. marcescens	without wetting agent	2	1 2					1 1		1 1	2 2
S. marcescens	with wetting agent	1	1	1							

* See Fig. 3 for penetration site code

Table 9

DISTRIBUTION OF PENETRATION SITES

Material:

2.0 mil Polypropylene A-22

Test Microorganism		Total No. of positive samples	Positive Replicate No.	Distribution of Penetration Sites							Total Sites
				aa*	a	b	c	d	e	f	
E. coli	without wetting agent	0									0
E. coli	with wetting agent	0									0
S. marcescens	without wetting agent	1	1							1	1
S. marcescens	with wetting agent	0									0

* See Fig. 3 for penetration site code

DISTRIBUTION OF PENETRATION SITES

Material:

3.0 mil Polypropylene A-22

Test Microorganism		Total No. of positive samples	Positive Replicate No.	Distribution of Penetration Sites							Total Sites
				aa*	a	b	c	d	e	f	
E. coli	without wetting agent	1	1					1			1
E. coli	with wetting agent	0									0
S. marcescens	without wetting agent	0									0
S. marcescens	with wetting agent	1	1							1	1

* See Fig. 3 for penetration site code

Table 11

DISTRIBUTION OF PENETRATION SITES

Material:

0.5 mil "A Mylar"/1.5 Polypropylene A-22
(Laminate)

Test Microorganism		Total No. of positive samples	Positive Replicate No.	Distribution of Penetration Sites							Total Sites
				aa*	a	b	c	d	e	f	
E. coli	without wetting agent	0									0
E. coli	with wetting agent	1	1					1			1
S. marcescens	without wetting agent	1	1	1							1
S. marcescens	with wetting agent										

* See Fig. 3 for penetration site code

TABLE 12

Abuse Test Results of Each Individual Pouch
Box No. 1
Materials: 0.5 "A Mylar"/0.35 Aluminum foil/1.5 mil PP-A-22

Pouch Number	Shipping Treatment	Carton Drop Treatment	Jacketed Drop Treatment	Static Load Treatment	Bio-Test	Type of failure (see TABLE 16)
1		x				B
2		x				B
3			x			C
4			x			C
5		x				D
6			x			C
7				x		D
8		x				B
9			x			C
10		x				E
11				x		C
12			x			C
13			x			B
14		x				E
15		x				F
16		x				F
17			x			B
18		x				E
19		x				C
20			x			C
21			x			E
22			x			E
23			x			E
24			x			C
25		x				E
26			x			C
27		x				B
28			x			D
29				x		C
30			x			C
31		x				B
32			x			C
33			x			C
34			x			B
35	x			x(r)		B
36	x		x(r)			C
37		x				D
38		x				D
39			x			D
40			x			D
41			x			D
42				x		B
43			x			D
44	x	x(r)				C
45			x			D
46			x			D
47			x			D
48			x			E
49			x			D
50		x				C
51		x				C
52			x			C
53			x			C
54			x			E
55			x			C
56	x		x(r)			C
57				x		D
58			x			C
59				x		D
60			x			C
61			x			C
62		x				B
63			x			C
64		x				B
65			x			F
66		x				C
67			x			C
68			x			C
69			x			C
70			x			F
71		x				F
72			x			D

(r) denotes a replacement pouch

TABLE 13

Abuse Test Results of Each Individual Pouch
Box No. 2
Materials: 0.5 mil "A Mylar"/0.35 mil Aluminum Foil/3.0 mil Polypropylene A-30

Pouch Number	Shipping Treatment	Carton Drop Treatment	Jacketed Drop Treatment	Static Load Treatment	Bio-Test	Type of Failure (see TABLE 16)
1			x			B
2				x		B
3			x			B
4				x		B
5				x		B
6		x				C
7		x				C
8		x				C
9		x				B
10			x			B
11			x			G
12		x				B
13			x			B
14			x			B
15			x			B
16			x			B
17			x			G
18			x			B
19			x			B
20			x			B
21			x			B
22			x			G
23			x			B
24			x			B
25		x				C
26			x			B
27			x			B
28		x				F
29			x			B
30		x				F
31			x			B
32				x		B
33		x				F
34			x			B
35			x			B
36				x		B
37		x				C
38			x			B
39			x			G
40		x				D
41			x			B
42		x				B
43		x				E
44			x			B
45			x			B
46		x				B
47		x				B
48			x			B
49		x				D
50			x			B
51		x				B
52			x			B
53		x				B
54			x			B
55		x				D
56		x				B
57		x				B
58		x				B
59		x				B
60		x				F
61		x				F
62		x				B
63		x				B
64		x				B
65		x				F
66			x			B
67			x			B
68		x				F
69		x				F
70			x			G
71		x				B
72		x				B

TABLE 14

Above Test Results of Each Individual Pouch
Run No. 3
Materials: 0.5 mil Polyester/0.35 mil Aluminum foil/2.0 mil vinyl

Pouch Number	Shipping Treatment	Carton Drop Treatment	Jacketed Drop Treatment	Static Load Treatment	Bio-Test	Type of failure (see Table 16)
1		x				H
2						
3				x		H
4						
5		x				H
6						
7						
8		x				F
9					x	C
10				x		F
11				x		L
12		x				F
13		x				G
14						
15						
16						
17						
18			x			G
19		x				H
20			x			F
21						
22						
23						
24						
25		x				G
26			x			H
27				x		A
28						
29			x			A
30						
31						
32			x			H
33						
34						
35			x			A
36		x				H
37						
38						
39			x			A
40		x				G
41						
42			x			A
43						
44						
45					x	H
46						B
47					x	B
48					x	B
49						
50				x		A
51						
52			x			A
53						
54						
55			x			H
56						
57						
58						
59						
60						
61						
62						
63						
64						
65			x			H
66						
67						
68						
69				x		H
70		x				H
71						
72			x			H

TABLE 35

Abuse Test Results of Each Individual Pouch
Box No. 4
Materials: 3.0 mil vinyl sealant (2s) 1.5 mil polypropylene sealant (2s)
3.0 polypropylene sealant (2s)

Pouch Number	Shipping Treatment	Carton Drop Treatment		Jacketed Drop Treatment	Static Load Treatment	Bio-Test	Type of failure (see TABLE 16)
		20 &	30 Drops				
1-V							
2-1.5		x		x(r)			B
3-3.0				x			D
4-1.5		x	x(r)				B
5-V							
6-3.0		x		x(r)			B
7-1.5				x			D
8-V					x		B
9-3.0			x				D
10-1.5		x		x(r)			B
11-V							
12-V							
13-1.5			x				C
14-V							
15-3.0			x				D
16-V							
17-3.0				x			C
18-1.5				x			B
19-3.0			x				D
20-V							
21-1.5		x			x(r)		C
22-3.0		x		x			B
23-V				x			B
24-1.5				x			B
25-1.5					x		C
26-V							
27-1.5			x				G
28-3.0		x	x(r)				C
29-V					x		B
30-1.5		x	x(r)				C
31-1.5					x		C
32-V							
33-3.0				x			E
34-3.0			x				E
35-3.0		x					D
36-1.5			x				B
37-3.0				x(r)			B
38-V							
39-1.5			x				D
40-3.0			x				D
41-V							
42-1.5					x		C
43-3.0		x		x(r)			B
44-V							
45-3.0			x				D
46-1.5			x				C
47-3.0		x			x(r)		C
48-V							
49-1.5				x			C
50-V							
51-3.0			x				D
52-1.5			x				C
53-V							
54-3.0			x				C
55-1.5				x			B
56-V							
57-3.0			x				D
58-1.5			x				B
59-V							
60-3.0			x				D
61-3.0		x			x(r)		C
62-1.5		x			x(r)		D
63-V		x					
64-3.0			x				B
65-V							
66-1.5				x			
67-3.0		x			x(r)		C
68-V					x		F
69-1.5				x			B
70-V							
71-1.5		x			x(r)		C
72-3.0			x				D

(r) denotes a replacement pouch.

TABLE 16

Description of Material Failure Resulting in Leakage of Pouches During Abuse

A. Failure due to fatigue occurring in the material caused by repetitive flexing.

B. Failure occurring in a three step sequence:

1. The inside layer (sealant layer) of the laminate breaking adjacent to the seal weld.

2. The aluminum foil and the outer layer delaminating adjacent to and away from the seal weld in the direction toward the center surface of the pouch.

3. Rupture of the aluminum foil and outer layer.

C. Failure occurring in the same manner as B, except that the delamination of the two outer materials occurred on top of the seal area.

D. Failure occurring in the same manner as C, except no rupture of material occurred. Delamination occurred along the entire seal depth.

E. Minute leakage occurring along the corner seal crease. The crease is the result of an overlapping of vertical and hortizontal seals at the corner edge of the pouch.

F. Rupture of the laminate not adjacent to the seal.

G. Side or top seal parted.

H. Rupture of the material adjacent to the seal.

TABLE 17

Abuse Test Results of the Jacketed Pouch Drop Treatment

Material: 0.5 mil "A Mylar"/0.35 mil Aluminum Foil/1.5 mil polypropylene

No.of Drops	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
No.of failures/drop	0	0	1	1	0	0	1	0	1	1	3	0	0	2	1	0	2	1	3	2	3	0	5	3	0	0	6	1	1	2
Pouch No.of failure			40	58			23		7		6			72	12		24	43	41	9	47		3	21			4	13	46	39
											42			35r			45		54	33	49		20	32			3?			53
											23								67		52		26	65			55			
																							60				56			
																							61				69			
																											70			

Material: 0.5 mil "A Mylar"/0.35 mil Aluminum foil/3.0 mil polypropylene

No.of Drops	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
No.of failures/drop	1	1	0	0	2	3	1	3	0	3	2	2	2	1	2	0	0	2	2	0	1	1	0	1	1	1	1	0	1	0
Pouch No.of failure	67	11			39	16	22	3		1	29	15	19	10	23			14	34		24	31		50	45	54	41		20	
					70	26		13		17	48	35	66		38			27	52											
						44		21		18																				

Material: 0.5 mil polyester/0.35 mil Aluminum foil/3.0 mil vinyl

No.of Drops	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
No.of failures/drop	1	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	1	0	2	0	1	0	0	2	0	1	0	0	2
Pouch no.of failure	32						20	29	39		37							72		18		55			52		32			35
																				26					65					42

Material: Pouches from Box #4 containing a composite of the three laminates listed above

No.of Drops	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
No.of failures/drop	0	0	1	0	0	0	1	0	0	2	0	2	1	1	1	0	2		1	1	1	0	0	0	0	0	1	1	1	2
Pouch no.of failure			33				34r			43r		24	23	22	17		7		3	2	36r						66	37r	69	6
										63r		49					10													29

"r" denotes replacement pouches

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author) Central Engineering Laboratories FMC Corporation Santa Clara, California	2a REPORT SECURITY CLASSIFICATION Unclassified 2b GROUP

3. REPORT TITLE

Resistance of Flexible Packaging Materials to Penetration by Microbial Agents

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final Report 21 June 65 - June 66

5 AUTHOR(S) (Last name, first name, initial)

Lampi, R. A.

6 REPORT DATE	7a TOTAL NO. OF PAGES	7b NO. OF REFS
April 1967	65	3

8a. CONTRACT OR GRANT NO. DA 19-129 AMC-662(N) b. PROJECT NO. 1M624101D552 c. Task 02 d.	9a. ORIGINATOR'S REPORT NUMBER(S) 9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) 67-62-GP

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	U. S. Army Natick Laboratories Natick, Massachusetts 01760

13 ABSTRACT

This project was conducted to study the resistance of flexible packaging materials used for thermally processed foods to penetration by microbial agents. Microbial agents were defined as bacterial cells being both aerobic and viable.

A procedure was evolved and equipment designed to study microbial penetration of flexible packaging materials.

The microbial penetration studies were made upon films, aluminum foils and laminates in sheet form. Creased films and laminates in sheet form and laminates in pouch form were subjected to abusive treatments.

Microbial penetration occurred only when pinholes were present in the materials. The 3-ply laminates approved for thermo processing when checked in sheet form, did not experience microbial penetration nor were pinholes present.

Creasing did not influence microbial penetration.

Laminates having polypropylene in their composition did not survive the abusive treatments.

DD FORM 1 JAN 64 1473

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14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Storage stability	8					
Containers	9		10			
Plastics	9		10			
Aluminum	9		10			
Laminates	9		10			
Flexible	0		0			
Military rations	4		4			
Protection			8			
Food			9			
Penetration			3			
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